iGEM Notebook

Made with Benchling

Project: Protocols

Authors: Kamila Drezek

Dates: 2016-05-19 to 2016-10-09

THURSDAY, 5/19/16

Preparing Reagants

SATURDAY, 5/21/16

- LB Broth (1L)
  - 20 grams per liter
- LB Agar (1L)
  - 40 grams per liter
- Prepare two liter containers of each- one per antibiotic (Cam and Carb)
- Autoclave for 1 hour

Preparing chloramphenicol stock (1000x)

- Final concentration of 1x chloramphenicol should be 25 ug/ml
- Stock concentration of 1000x chloramphenicol should be 25 mg/ml
- Prepare 50 ml of stock to aliquot into microcentrifuge tubes by measuring 1.25 g of chloramphenicol and ~50ml of 200 proof ethanol in a 50 ml Falcon
- Filter chloramphenicol through 20 um syringe filter

Preparing Plates

- 20ml agar per plate

Innoculate Vector 1721

- 5 ml of LB+carb
- Stab cell stock
- Grow overnight at 37C shaker

SUNDAY, 5/22/16

- Preparing Cell stock of 1721
  - Box Name- iGEM 2016 Source Box
  - Freezer Number- 3
  - Shelf- 3
- Vector 1721 is miniprepped
  - See protocol sheet
- Vector 1721 is digested (300ul digest)(1-2hrs)
  - 24000 ng digestion of DNA
    - 30 ul buffer (cold room)
    - 9ul of BpsI (-80C, small green box)(incubation in 37C)
    - 101 ul of water
- Digestion Clean-Up of Vector 1721
  - See PCR cleanup protocol
  - Note- vector was eluted in 50 ul of EB not 30 ul.
- Dilution of Oligos (ideal final concentration is 100uM)
  - Short Cut-
    - 300 ul of TE for values ~25- ~35 nmol
    - 200 ul of TE for value ~15 - ~24 nmol
  - Note- Oligo 12, 13, 14, 17, 18, and 20 was vortex without liquid initially
- Annealing of pGEX oligos
  - See protocol
  - Annealing time- 2 minutes

dilute oligos to 1ng/ul

Ligation of 1721 and insert

Note- diluted annealed oligos named- pGEX#A where # is the number of the construct

24 reactions

139.2 ul of water

24 ul of T4 Ligase

24 ul of T4 Ligase Buffer

26.4 ul of Vector

Transformation

See protocol

Plates put in incubator at 7:09 PM- Desired pick up time is 10:09

MONDAY, 5/23/16

pGEX plates were pulled from incubator at 10:15

Growth and density of all samples exceeded that of the negative control

TUESDAY, 5/24/16

Picked two colonies per plate and grew in 4ml of LB+carb in 37C shaker.

Created stab plate for each colony.

WEDNESDAY, 5/25/16

Minipreping colonies

Notes: During the miniprep samples from colony 10 were contaminated

Test Cut

4 ul DNA

1 ul 2.1

.25 of ul each enzyme (Bsp1 and Nco1)

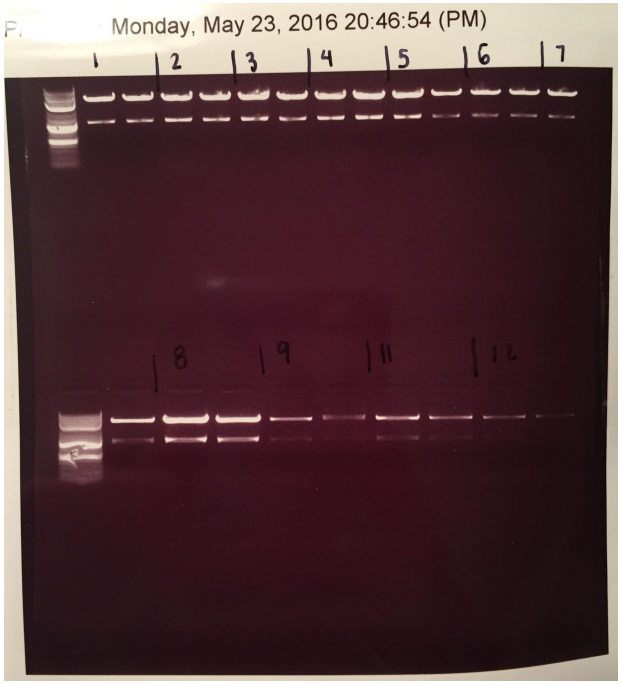
4.5 ul water

Run digestion for at least 15 minutes

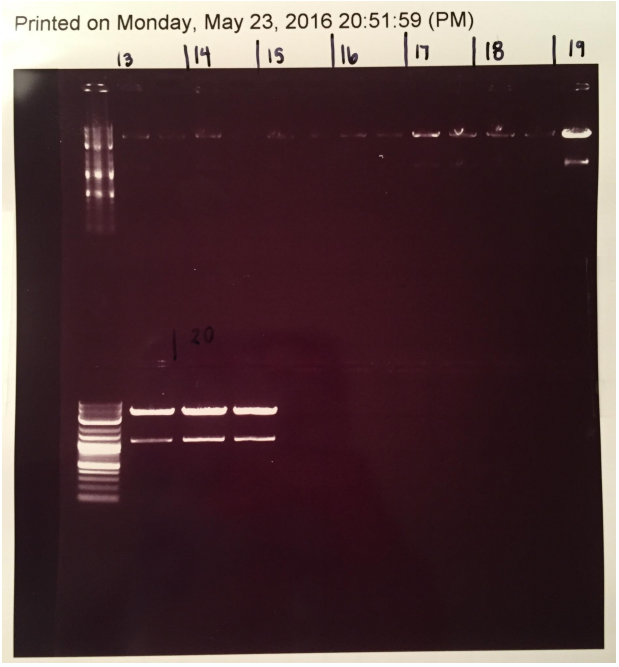
Test Cut Results

All colonies appeared with proper bands at 1.1 kb and 4.2kb and no bands at 440 bp. We decided to send only the first colony from each set in for sequencing

pGEX1-1 to pGEX12-2 test cuts



pGEX13-1 to pGEX20-2 test cuts



THURSDAY, 5/26/16

Sequencing results are in

All sequences with the exceptions of pGEX1-1, pGEX14-1, and pGEX 15-1 were successful.

All sequences reamining sequences were then grown up for cell stock

Note: While pGEX 6-1 sequenced properly the tube containing its reference appeared to have been contaminated and pGEX 6-2 will be sent in for sequencing to reverify

Sequencing sent in

pGEX: 1-2, 6-2, 14-1, 14-2, 15-1, 15-2, 10-1, 10-2

Restriction digest of 2490 and DC16 with MluI and NruI

5ul buffer3.1

6ul DNA

1.5ul Nru1

1.5ul Mlu1

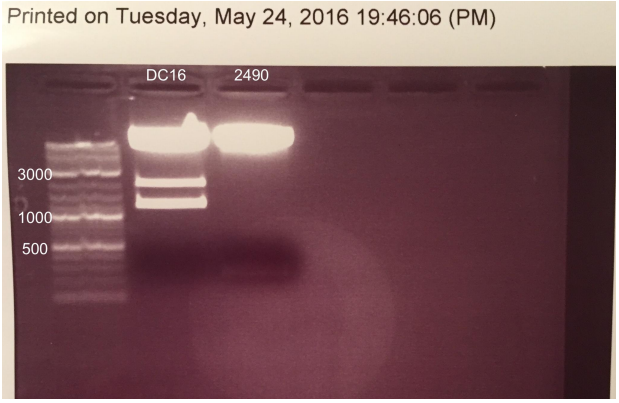
36ul water

Prerequisites to subvectors which will be used to create one plasmid with Cre and both Flps

Subvectors to be made have homology with each other, allowing for easy gibson assembly with no PCR (promoters and ERT2 - modified estrogen receptor - have repeat regions)

Digestion of DC16 yielded 3 bands rather than the expected 2. Culprit may be unreliable cell stock.

DC16 2490 1st digestion.jpg



We were supposed to have: DC16 bands at 10723bp, **1172bb**.

2490 bands at **10590bp** and 227bp.

Ligate 2490 and DC16 to create 2534 (10ul)
- See Ligation Calculator
- 4.2 ul DI water
- 1.0 ul DC16 (insert)
- 2.8 ul 2490 (vector)
- 1 ul T4 Ligase Buffer
- 1 ul T4 Ligase

Construct 2534 and a negative control are transformed

Tissue Culture Room
- Prepping hood for trannsfection
  - Remember to clean the hood down with 70% ethanol
- Aspirate all media from the plate to aid in the trypsin function
  - Kidney cells are stored in T75 flask
- After aspiration perform a PBS wash
  - Phosphate Buffer Solution- a salt water solution (~5ml wash)
- Trypsin is used remove kidney cells from the plate.
  - Add 2.5ml of Trypsin to the T75
    - Dont shoot directed at the cells, let the trpysin run over them.
- Addition of Medium will neutralize trypsin
  - Add 7.5 ml of media to plate
  - And tritrate (Pipette up and down)
  - To improve cell count- spin cells down then respend them
    - Spin at 300 RCF for 2-5 minutes
    - To resuspend- tap the hood with the 50ml falcon tube
    - Add back 10 ml of media and tritrate
- Performing a cell count
  - Add 10 ul of cell solution to a cell counting slides
    - Divide the cell count concentration by 2
    - Actual Cell Counts
      - ~2.5 Million cells per ml
- Magic Number for transfection:
  - 200,000 cells per ml
  - For each T75- 20 ml of solution
  - For desired concentration of cells-
    - Add 4.8 ml of cell concentrate
    - Add 55.2 ml of media
  - Continuing the cell line
    - Note:
      - Split/Passage: diluting cells in the same vessel
    - Split the parent cell line

FRIDAY, 5/27/16

pGEX Cells moved to Stock
- 1-13, 16-20

Sequencing results
- 6-2 and 1-2 are perfect
- pGEX 14 ans 15 failde to sequence again

Transformation for Construct 2534
- No significant growth in the positive- less than what appeared in the negative -> failure

Digest a different aliquot of DC16 at a low concentration
- Need 4ng of DNA with DNA at 45ng/uL so need 89 uL of DNA; will do 100 ish uL digest

89 uL DNA
1.5 uL MluI
1.5 uL NruI
10 uL Buffer 3.1
No water

Ligation of dc16 insert and 2490 vector
2.7 ul of vector
1.7 ul of insert
3.5 ul of water
1 ul of T4 Ligase
1 ul of T4 Ligase Buffer

Digestion of pSb1A3 and pBS1C3
40 ul of DNA
5 ul of Cutsmart BUffer
1.5 ul of EcoRI
1.5 ul of PstI
2 ul of Water
After digestion treat witl DnpI for 15 minutes
PCR Clean- up and elute in 30 ul of EB

Gibson of pSb1A3 (2533) and pSb1C3 (2531)

CONSTRUCT NAME 2531
Mass Vec =25
Ratio = 3
V A B C D W
SIZE 2000 1200 600 0 0
CONC 5.6 10 10 1 1
VOL 4.5 4.5 2.3 0.0 0.0 -1.2
CONSTRUCT NAME 2533
SIZE 2000 1200 600 0 0
CONC 23 10 10 1 1
VOL 1.1 4.5 2.3 0.0 0.0 2.2

Both the Gibson Assembly Transformation and the Ligation Transformation are over 10kb
See Protocol
Stable Line Integration of 2494-2496
See Protocol for Stable Line Transfection
Construct 900- Piggy back transpose
Cell status preceeding transfection
Low cell count ~30-40% confluences
Thaw out 2 tubes of PEI
Use an incubator in a BL2 room
Note: We are making 4x the number of cells than what is required
Mixes
DNA part
.78 ul of transposase
3.2 ul of DNA
.15 M NaCl (3ml per tube)

PEI part
2ml of PEI
.15 M NaCl (10.5 ml)

Add 4 ml of PEI part to DNA part

Transfection

Don't pour directly onto the- shoot away from the cells

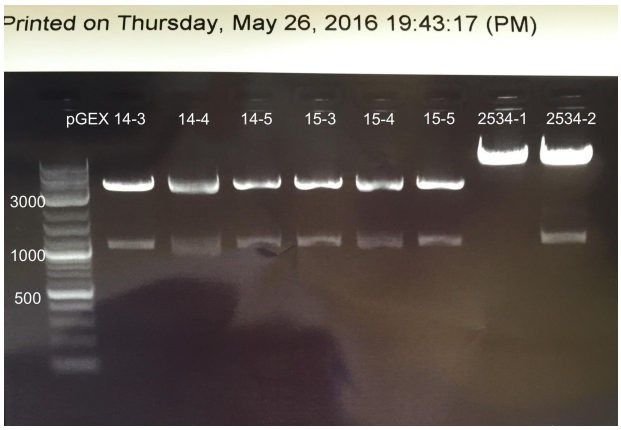
SATURDAY, 5/28/16

Create cell stock of pGEX10-1

MIniprep of 14-3/4/5 15-3/4/5 and 2534-1/2

Test Cut Results

05-26-16 pGEX14,15 and 2434 test cuts.jpeg



pGEX 14-3, pGEX 15-5, 2534-2 => sent to sequencing

Cell inspection preceeding puromycin selection

~90% confluence

Puromycin Selection

4ul of concentrated puromycin - 2ug per ml to each flask

Wait 10 days for total transient plasmids are diluted outs.

SUNDAY, 5/29/16

Sequencing Results

pGEX 15-5 can be moved to cell stock

pGEX 14-3 failed

2534 failed

Sent Sequencing

pGEX 14-4, pGEX 14-5

Digestion

2531

4 uL DNA

4 uL DI

1 uL CutSmart

0.5 uL EcoRI and AgeI

2533

4 uL DNA

4 uL DI

1 uL CutSmart

0.5 uL EcoRI and AgeI

DC16 midiprep

1 uL DNA at 1:10 dilution

7 uL DI

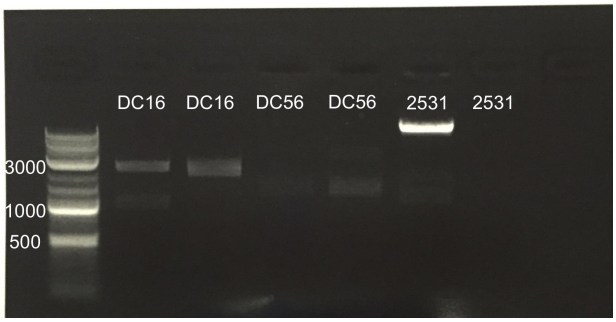
1 uL Buffer 3.1

0.5 uL MluI and NruI

DC16 miniprep
- 2 uL DNA
- 6 uL DI
- 1 uL Buffer 3.1
- 0.5 uL MluI and NruI

05-27-2016 DC16 DC56 2531 test cut.jpeg

Printed on Friday, May 27, 2016 19:33:43 (PM)



dc16 Primers
BP0165, BP0162 reverse
BP0198, BP0176 forward
dc56 Primers
BP0165, BP0130 reverse
BP0038, BP0188 forward

pGEX 14-4 and pGEX14-5 moved to cell stock pending sequencing

TUESDAY, 5/31/16

Phosphorylating and Annealing Oligos for pGOP
Moved Construct 2531 to cell stock
Grow up more 2531 for ligation
- Because of Chloramphenicol's effect on growth, we will grow this up for 24 hours

Oligos are at 1000x concentration- dilute to 1x (~1ng/ul)
Picked more colonies from 2533 and grew up overnight

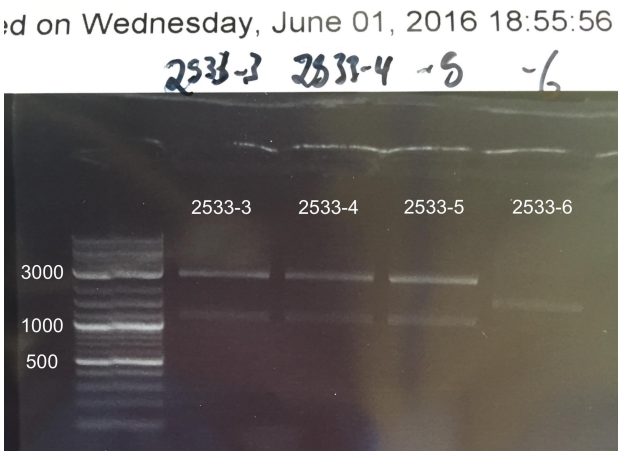
WEDNESDAY, 6/1/16

Miniprep 2531 (grown again due to low inital concentration) and 2533 (initial sequencing was incorrect)
Digest 2531 with BbsI (50 uL total)
- 30 uL DNA
- 5 uL Buffer 2.1
- 2 uL BbsI
- 13 uL DI water

Ligate oligos with 2531 to create pGOP1-20 (10uL total)
- See protocol
- 0.4 uL 2531 (BbsI)
- 1.3 uL annealed oligos
- 6.4 uL DI water
- 1 uL T4 Ligase Buffer
- 1 uL T4 Ligase

All pGOP plasmids were transformed and plated (25 ul) with Control
Test Cut 2533 with EcoR1 Age1

06-01-2016 testcut of 2533.jpeg

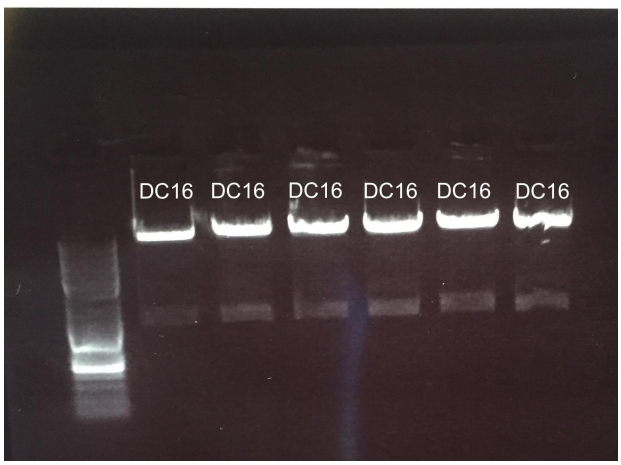


2533-3/4/5 were sent in for sequencing
Prepped for creation of comp cells for tomorrow
    Mix 25g PEG8000 with 30ml water and disolve over night

THURSDAY, 6/2/16

Pick 2 colonies for each pGOP
Miniprepped dc16 and dc56
Prepared 200ml TSS Solution
dc 16 test cut with MluI and NruI
    expected bands: 10723, 1172

06-02-2016 DC16 testcuts.jpeg



DC16: 1,2,3,5 were good

dc 56 test cut with AgeIHF and EcoRIHF
    expected bands: 6700, 1000

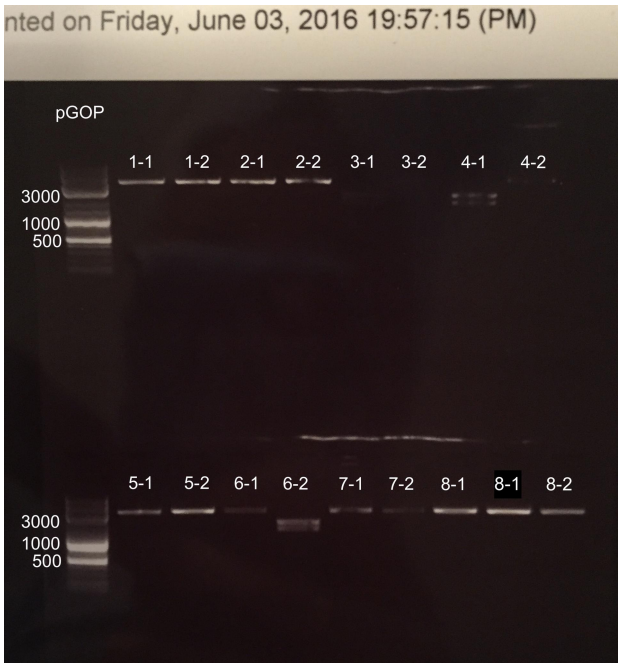
Primer 0170 for 2533 failed and this impeded the sequencing
Preparing Top10 comp cells
    First OD (optical density) measurement: 0.2 (time: 2:15 p.m.)
    Second OD measurement: 0.34 (time 2:45 p.m.)
    Third OD measurement: 0.419 (time 3:05 p.m.)
    Fourth OD measurment: 0.486 (time 3:35 p.m.)

FRIDAY, 6/3/16

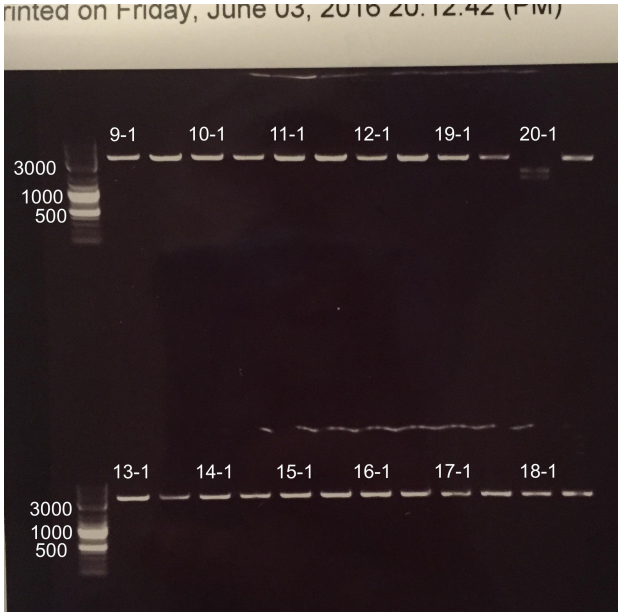
miniprepped pGOP plasmids

test cut pGOP plasmids with Bbs1 and Spe1

06-03-2016 pGOP testcuts 1-8.jpeg



06-03-2016 pGOP9-20 testcuts.jpeg



grew up more _____ for midiprep

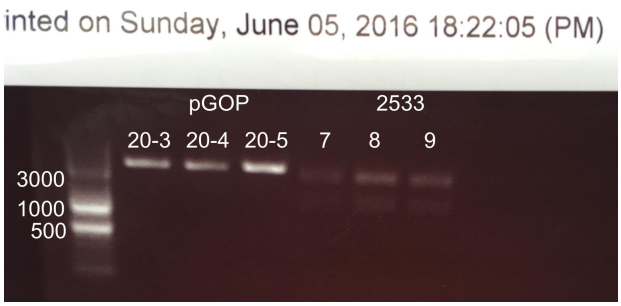
SATURDAY, 6/4/16

Picked three colonies of pGOP 20 (3, 4, and 5)
Grew up pGOP 20-3/4/5
Miniprep 2533-7/8/9

SUNDAY, 6/5/16

Miniprep 20-3/4/5
Test Cut for 2533 and pGOP 20 addition colonies

06-05-2016 pGOP20 and 2533 test cuts.jpeg



MONDAY, 6/6/16

Miniprep pGOP20-R1/R2/R3
Test cuts for pGOP20-R1/R2/R3

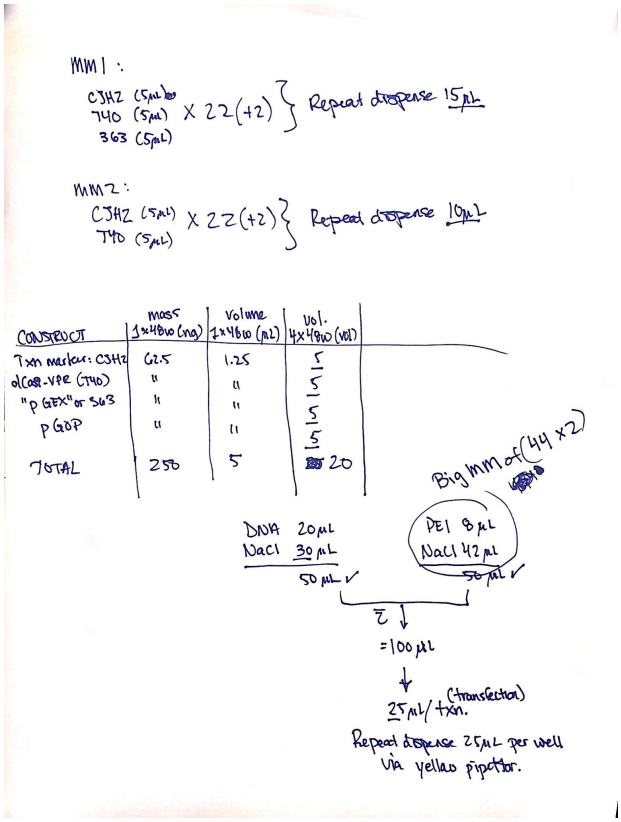
Cell Stock pGOP 1-19
Grew up DC16-1 and DC56-1 for cell stock
Sent pGOP 20-3/4/5 and 2533-7/8/9 for sequencing
Diluted pGEX 1-20 and pGOP 1-19 to 50 ng/uL
Made a liter of LB + Carb
Nanodropped pGEX 1-20 and pGOP 1-19
    Inoculated pGEX16-1 due to low concentration

Midiprep for Transfection
    Prepared 150 mL of LB + Carb with pCJH2
    Prepared 150 mL of LB + Carb with T40
    Prepared 150 mL of LB + Carb with pBW363

TUESDAY, 6/7/16

Plan for transfection of pGEXs and pGOPs to be performed on Thursday

new doc 7_1.jpg



Miniprep of pGOP12, pGEX16, DC174 and DC100 (both DCs will be used as positive controls transfection on Thursday)
Midiprep of pBW363 (Blank), T40 (dCas9), pCJH2 (BFP transfection marker), DC16 and DC56
    Evaporated supernatant

WEDNESDAY, 6/8/16

Cell stocked pGOP 20
50ul Restriction digest of 2490 and DC16 with MluI and NruI to create 2534
    5ul buffer3.1
    6ul DNA
    1.5ul Nru1
    1.5ul Mlu1
    36ul water
    incubate 1 hr in 37C
    Got 3 bands so threw out digest and are now investigating our enzymes to see if there has been contamination
Stocked midiprep DNA with 1x TE
Grew up T40 for further minipreps
Prepared HEK293 plates for tomorrow's transfection
    Add 250ul of 200000cells/ml cell solution to each well on 48 well plate

THURSDAY, 6/9/16

Transfected HEK cells

Screen Shot 2016-08-01 at 12.18.39.png

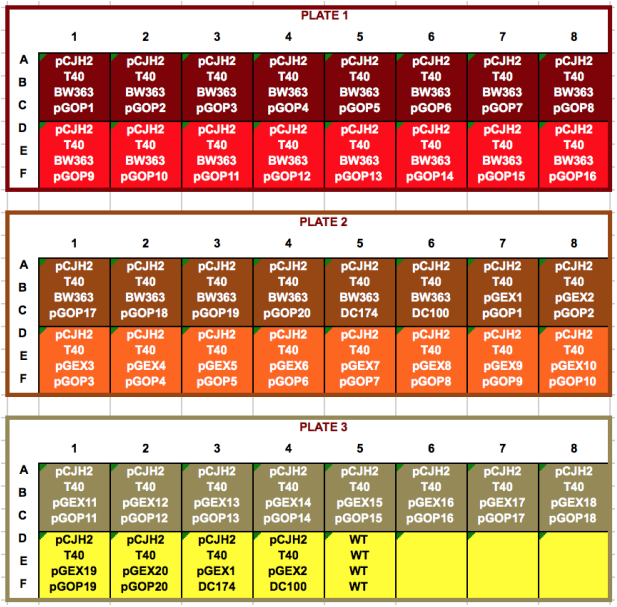


PLATE 1

	1	2	3	4	5	6	7	8
A	pCJH2 T40 BW363 pGOP1	pCJH2 T40 BW363 pGOP2	pCJH2 T40 BW363 pGOP3	pCJH2 T40 BW363 pGOP4	pCJH2 T40 BW363 pGOP5	pCJH2 T40 BW363 pGOP6	pCJH2 T40 BW363 pGOP7	pCJH2 T40 BW363 pGOP8
B								
C								
D	pCJH2 T40 BW363 pGOP9	pCJH2 T40 BW363 pGOP10	pCJH2 T40 BW363 pGOP11	pCJH2 T40 BW363 pGOP12	pCJH2 T40 BW363 pGOP13	pCJH2 T40 BW363 pGOP14	pCJH2 T40 BW363 pGOP15	pCJH2 T40 BW363 pGOP16
E								
F								

PLATE 2

	1	2	3	4	5	6	7	8
A	pCJH2 T40 BW363 pGOP17	pCJH2 T40 BW363 pGOP18	pCJH2 T40 BW363 pGOP19	pCJH2 T40 BW363 pGOP20	pCJH2 T40 BW363 DC174	pCJH2 T40 BW363 DC100	pCJH2 T40 pGEX1 pGOP1	pCJH2 T40 pGEX2 pGOP2
B								
C								
D	pCJH2 T40 pGEX3 pGOP3	pCJH2 T40 pGEX4 pGOP4	pCJH2 T40 pGEX5 pGOP5	pCJH2 T40 pGEX6 pGOP6	pCJH2 T40 pGEX7 pGOP7	pCJH2 T40 pGEX8 pGOP8	pCJH2 T40 pGEX9 pGOP9	pCJH2 T40 pGEX10 pGOP10
E								
F								

PLATE 3

	1	2	3	4	5	6	7	8
A	pCJH2 T40 pGEX11 pGOP11	pCJH2 T40 pGEX12 pGOP12	pCJH2 T40 pGEX13 pGOP13	pCJH2 T40 pGEX14 pGOP14	pCJH2 T40 pGEX15 pGOP15	pCJH2 T40 pGEX16 pGOP16	pCJH2 T40 pGEX17 pGOP17	pCJH2 T40 pGEX18 pGOP18
B								
C								
D	pCJH2 T40 pGEX19 pGOP19	pCJH2 T40 pGEX20 pGOP20	pCJH2 T40 pGEX1 DC174	pCJH2 T40 pGEX2 DC100	WT WT WT WT			
E								
F								

Miniprepped T40

Diluted pCJH2, pBW363, and T40 for transfection

Checked and rediluted pGEXs and pGOPs for transfection

note- pGEX11 is contaminated

pGEX7 is too low of concentration

FRIDAY, 6/10/16

Checked transfection plates under a microscope

all worked; however, Cre showed leakiness

Resent DC16-1 for sequencing due to QuintaraBio sequencing faillure

Passaging HEK293 cells

MONDAY, 6/13/16

Miniprepped pGOP1-20 (inoculated in Cam last night)

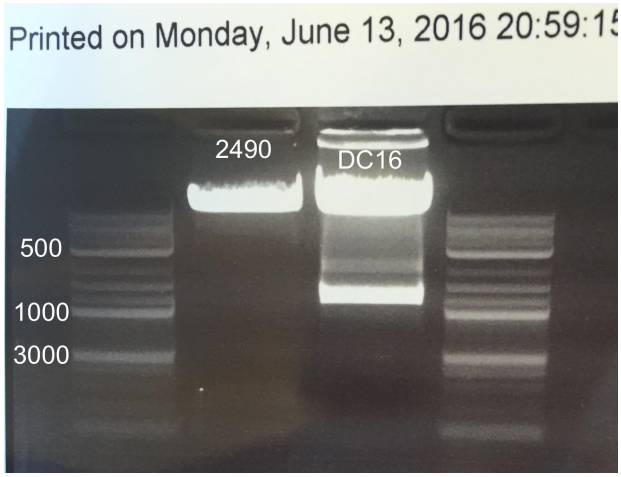
Cut DC16 and 2490 with MluI and NruI

- 8 uL DC16 or 11uL of 2490
- 1.5 uL MluI
- 1.5 uL NruI
- 5uL buffer 3.1
- 34 uL water or 31 uL water (2490)

Run Gel Extraction on 2490 and DC16

- Bands
- DC16
  - 10723
  - 1172 - WANTED BAND
- 2490
  - 10590 - WANTED BAND
  - 227

06-13-2016 dc16 2490.jpeg

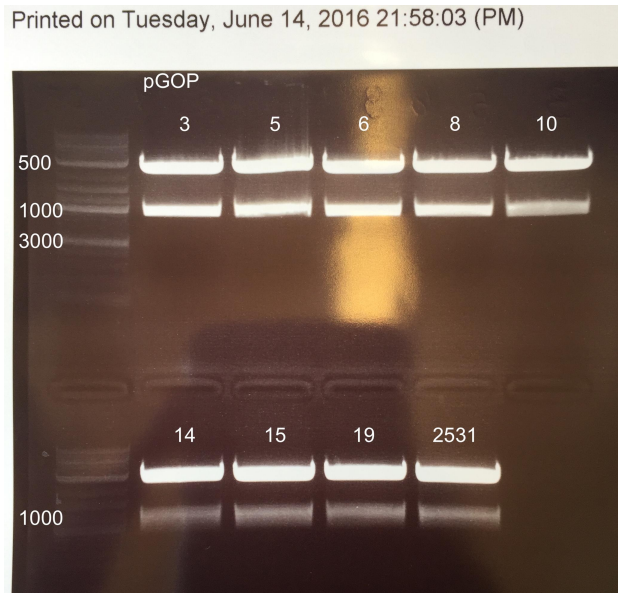


Ligation of 2490 and DC16 to make 2534
Ligations were transformed and plated

TUESDAY, 6/14/16

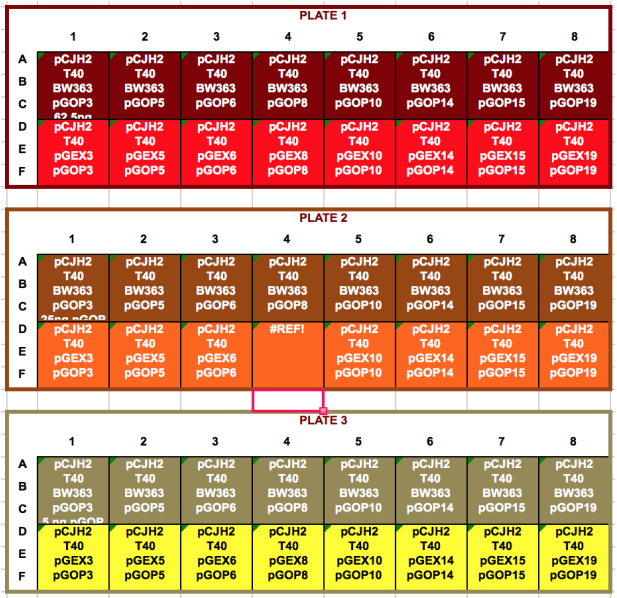
Diluted primers for PCR reaction of pGPX1 (2531)
PCR GFP, BFP and mRuby
Digested pGOPs 3,5,6,8,10,14,15,19 and pGPX1 with Mlu1-HF and Age1-HF in preparation of making pGOPs with GFP, BFP and mRuby

06-14-2016 pGOPs.jpeg



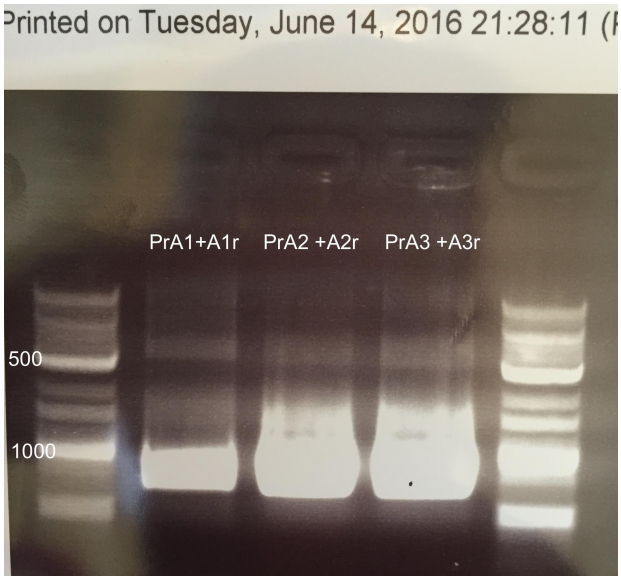
Miniprep 2531
Picked colonies for 2534
Transfected HEK cells with different concentrations of the 8 pGOPs given above, to test whether the concentration of pGOPs in the cell was causing high levels of basal expression)
Transfected transfection marker, T40 (dCas9) pGEXs, Blank plasmid and pGOP. pGOP at 62.5, 30 and 7.5 ng

Screen Shot 2016-08-01 at 12.17.24.png



PCR digested GFP, BFP and mRuby with Mlu1-HF and Age1-HF

06-14-2016 Primers.jpeg



WEDNESDAY, 6/15

Miniprepped T40 (was running low)

Ligated GFP, BFP and mRuby into pGOP 3,5,6,8,10,14,15,19. Transformed onto Cam plates

THURSDAY, 6/16

Ran FACs on transfection from Tuesday

PCR the silencer out of DC100 (has UAS and pG5) to put into pGOPs and pGPX6 with PrA4 and PrA4r

Digested DC 100 and prepared annealed oligos o83-o122 to prepare _____

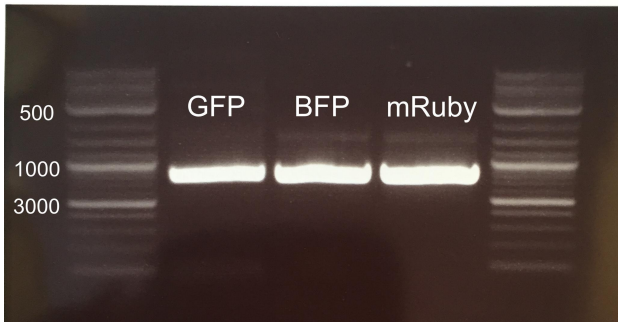
FRIDAY, 6/17

Miniprepped Transformed plasmids from 6-15-16

PCR for GFP, BFP, and mRuby

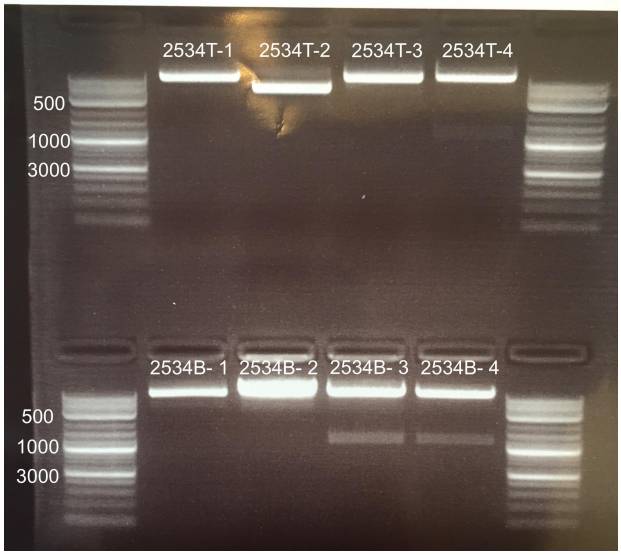
06-17-2016 GFP BFP mRuby.jpeg

Printed on Friday, June 17, 2016 19:20:27 (PM)



06-17-2016 2534t and 2534b.jpeg

rinted on Thursday, June 16, 2016 16:45:05 (PM)

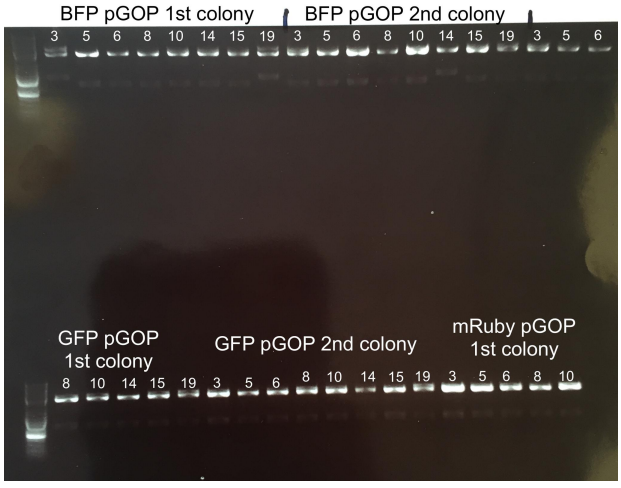


Sent plasmdis in for sequencing

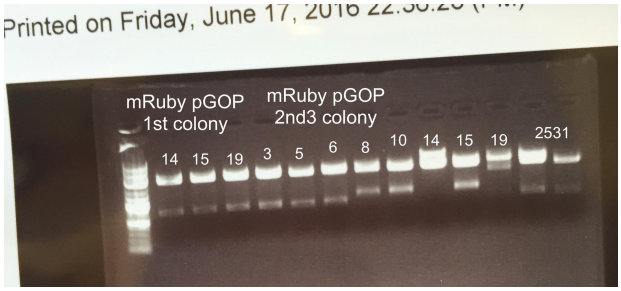
Sequence for 2534B-4 is verified as functioning and will be moved forward with cloning

06-17-2016 bfp gfp pgops.jpeg

rinted on Friday, June 17, 2016 22:34:58 (PM)



06-17-2016 mRuby pGOPs and 2531.jpeg



MONDAY, 6/20

Cell Stocked plasmids for BFP, GFP, and mRuby that sequenced properly
Miniprepped plasmids
Picked colonies for mRuby pGOP 10,14, and 15

TUESDAY, 6/21

Cell stocked BFP pGOP 15
Picked colonies for GFP pGOP 8, GFP pGOP 19, mRuby pGOP 10, mRuby pGOP 15, and mRuby pGOP 19
Miniprepped plasminds from 6-20-16 (mRuby pGOP 10-3, 10-4, 14-3, 14-5, 14-6, 15-3)
Sent mRuby pGOP 14-3/4/5 and BFP pGOP 19-2 for sequencing
Passaged new HEK cells to P7
Made 2 new bottles of D5 Media (D5= 5%FBS)
- 500ml of DMEM base
- 25ml FBS (alloquoted in fridge across hallway from TC room)
- 5ml L-Glut
- 5ml P/S
- 5ml sodium pyruvate

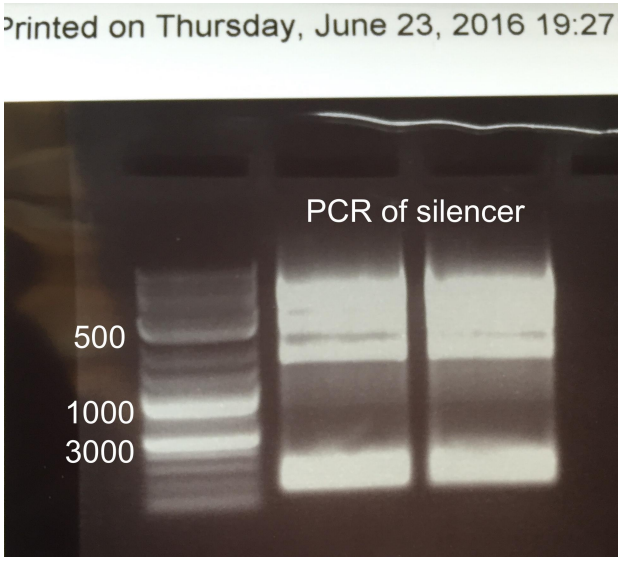
WEDNESDAY, 6/22

Cell stocked picked colonies from 6/21/16 (GFP pGOP 8, GFP pGOP 19, mRuby pGOP 10, mRuby pGOP 15, and mRuby pGOP 19).

THURSDAY, 6/23

Gel extract PrA4/4r (silencer)

06-23-2016 PCR of silencer.jpeg



Digested DC100 with NheI and dropped in annealed oligos with ligation to make pDOP 1-20 (backbone from DC100 with gRNAs dropped in)
sent pGOP 6-9 back in for sequencing - suspected error in gRNA target site

MONDAY, 6/27

ligate and transform pDOP 1-20 (DC100 and annealed oligos)
inoculate pGPX1 so we can make pGPX2 and 3

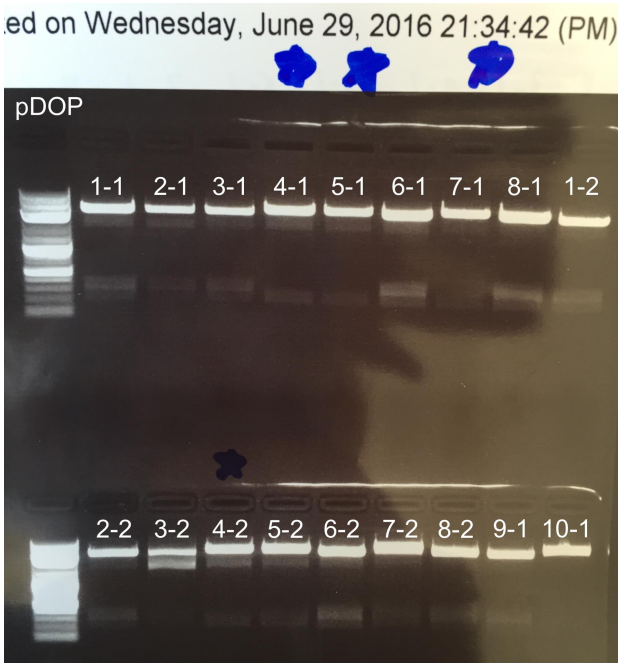
TUESDAY, 6/28

Prepped for transfextion
Picked pDOP1-20
Gel extracted pGPX1

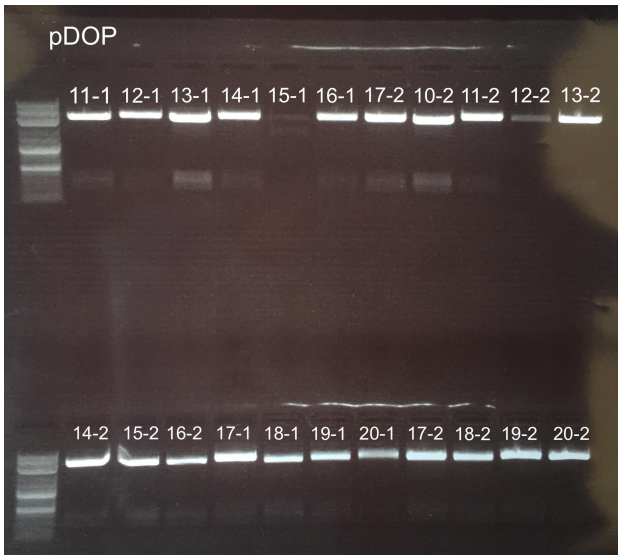
WEDNESDAY, 6/29

ligate and transfrom pGPX2 and pGPX3 (pGPX1 MluI Age1 with PrA1/1r and PrA2/2r Mlu1 and Age1)
miniprep pDOP 1-20
Test Cut pDOP1-20 with NheI-HF and StuI

06-29-2016 pDOP 1-10.jpeg



06-29-2016 pDOP 11-20.jpeg



Transfected pGOP 3,5,6,8,10,14,15,19 of iRFP, BFP, GFP, and mRuby

Screen Shot 2016-08-01 at 12.16.09.png

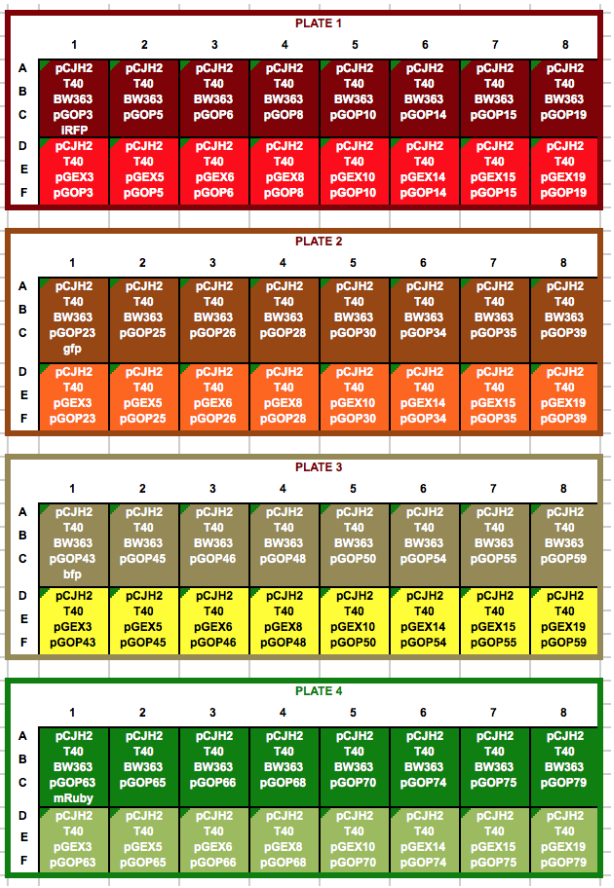


PLATE 1

	1	2	3	4	5	6	7	8
A	pCJH2 T40 BW363 pGOP3 iRFP	pCJH2 T40 BW363 pGOP5	pCJH2 T40 BW363 pGOP6	pCJH2 T40 BW363 pGOP8	pCJH2 T40 BW363 pGOP10	pCJH2 T40 BW363 pGOP14	pCJH2 T40 BW363 pGOP15	pCJH2 T40 BW363 pGOP19
B								
C								
D	pCJH2 T40 pGEX3 pGOP3	pCJH2 T40 pGEX5 pGOP5	pCJH2 T40 pGEX6 pGOP6	pCJH2 T40 pGEX8 pGOP8	pCJH2 T40 pGEX10 pGOP10	pCJH2 T40 pGEX14 pGOP14	pCJH2 T40 pGEX15 pGOP15	pCJH2 T40 pGEX19 pGOP19
E								
F								

PLATE 2

	1	2	3	4	5	6	7	8
A	pCJH2 T40 BW363 pGOP23 gfp	pCJH2 T40 BW363 pGOP25	pCJH2 T40 BW363 pGOP26	pCJH2 T40 BW363 pGOP28	pCJH2 T40 BW363 pGOP30	pCJH2 T40 BW363 pGOP34	pCJH2 T40 BW363 pGOP35	pCJH2 T40 BW363 pGOP39
B								
C								
D	pCJH2 T40 pGEX3 pGOP23	pCJH2 T40 pGEX5 pGOP25	pCJH2 T40 pGEX6 pGOP26	pCJH2 T40 pGEX8 pGOP28	pCJH2 T40 pGEX10 pGOP30	pCJH2 T40 pGEX14 pGOP34	pCJH2 T40 pGEX15 pGOP35	pCJH2 T40 pGEX19 pGOP39
E								
F								

PLATE 3

	1	2	3	4	5	6	7	8
A	pCJH2 T40 BW363 pGOP43 bfp	pCJH2 T40 BW363 pGOP45	pCJH2 T40 BW363 pGOP46	pCJH2 T40 BW363 pGOP48	pCJH2 T40 BW363 pGOP50	pCJH2 T40 BW363 pGOP54	pCJH2 T40 BW363 pGOP55	pCJH2 T40 BW363 pGOP59
B								
C								
D	pCJH2 T40 pGEX3 pGOP43	pCJH2 T40 pGEX5 pGOP45	pCJH2 T40 pGEX6 pGOP46	pCJH2 T40 pGEX8 pGOP48	pCJH2 T40 pGEX10 pGOP50	pCJH2 T40 pGEX14 pGOP54	pCJH2 T40 pGEX15 pGOP55	pCJH2 T40 pGEX19 pGOP59
E								
F								

PLATE 4

	1	2	3	4	5	6	7	8
A	pCJH2 T40 BW363 pGOP63 mRuby	pCJH2 T40 BW363 pGOP65	pCJH2 T40 BW363 pGOP66	pCJH2 T40 BW363 pGOP68	pCJH2 T40 BW363 pGOP70	pCJH2 T40 BW363 pGOP74	pCJH2 T40 BW363 pGOP75	pCJH2 T40 BW363 pGOP79
B								
C								
D	pCJH2 T40 pGEX3 pGOP63	pCJH2 T40 pGEX5 pGOP65	pCJH2 T40 pGEX6 pGOP66	pCJH2 T40 pGEX8 pGOP68	pCJH2 T40 pGEX10 pGOP70	pCJH2 T40 pGEX14 pGOP74	pCJH2 T40 pGEX15 pGOP75	pCJH2 T40 pGEX19 pGOP79
E								
F								

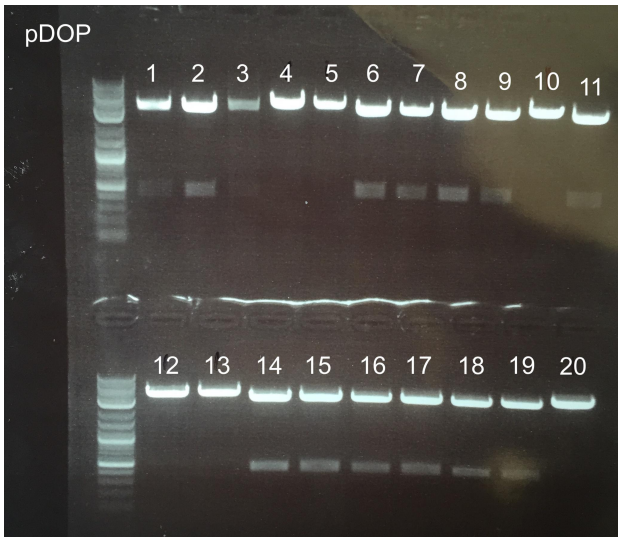
THURSDAY, 6/30

PIcked pGPX2 and pGPX3 colonies

Reannealed-phosphorylated- ligated - and transformed pDOPs

recut pDOPs

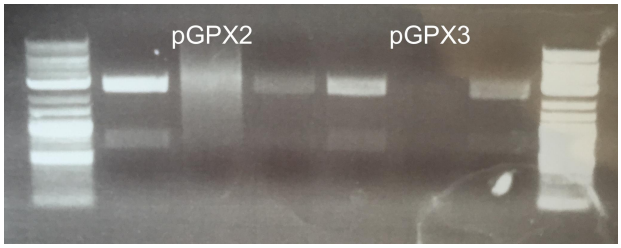
06-30-2016 recut pDOPs.jpeg



FRIDAY, 7/1

Miniprep of GPX 2-1/2/3 and GPX 3-1/2/3
Test-cut GPX 2 and 3
Picked colonies and grew up pDOP 1-4; 6-11; 14-20
Ran FACS (flow cytometry)
    Did not test BFP due to wrong transfection marker
PCR clean up of 2485; 2487; and 2489 with NotI and Cip
Gel extraction of 2484, 2486, 2488, and 2534 digested with AscI and NotI

07-01-2016 pGPX2 3.jpeg



SATURDAY, 7/2

Miniprep and test cut pDOP 1-4; 6-11; 14-20 colonies 1 and 2
    digested with NheI and PstI
Vectors pGPX2 and pGPX3 are sequenced verifeid. pGOP 66,34, and 26 could not be sequenced properly (quintara error)
Reenoculate pGEX and GFP pGOP 3, 5, 6, 8, 10, 14, 15, 19 gh54

SUNDAY, 7/3

Miniprep pGEX and GFP pGOP 3, 5, 6, 8, 10, 14, 15, 19
Gibson and transform pREC 1, 2, 3

MONDAY, 7/4

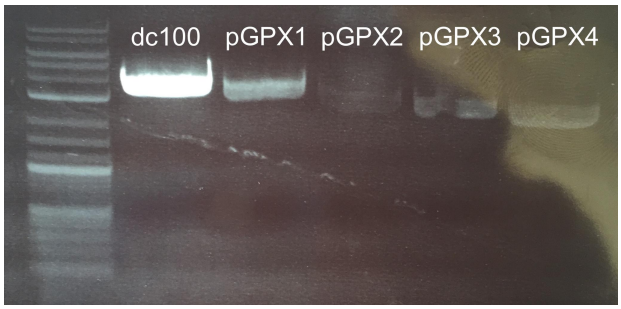
Passage HEK cells
Inoculate pREC 1, 2, 3

TUESDAY, 7/5

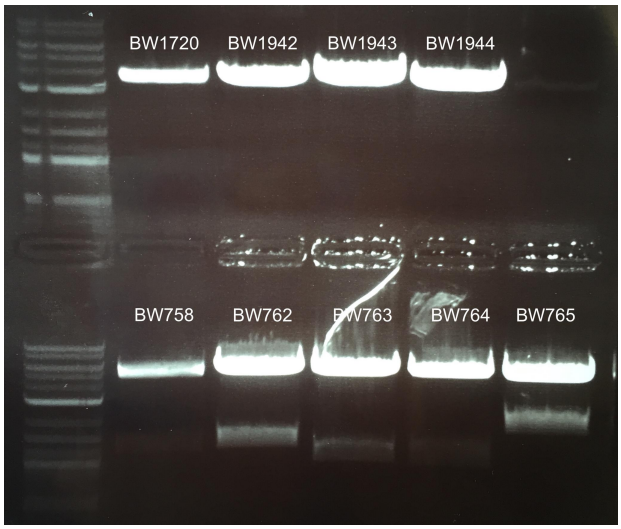
Miniprep pREC 1, 2, 3
Enoculate BW1720
Digested BW1720, and BW1942-43 with BbsI
Digested BW758, and BW762-65 with AgeI and EcoRI

Digested DC 100 with NheI and Cip
Digested pGPX1-4 with Xbha and Bbsi

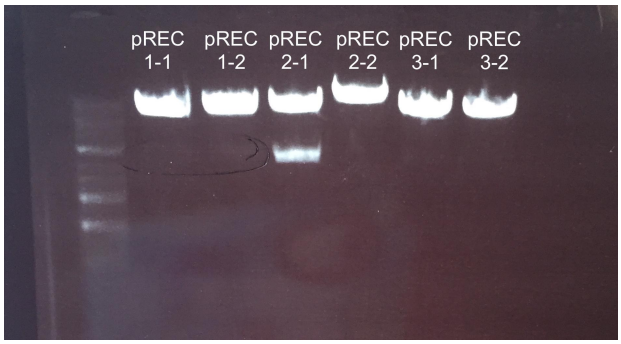
07-05-2016 dc100, pGPX1:2:3:4.jpeg



07-05-2016 BW contstructs.jpeg



07-05-2016 pREC.jpeg



Transfected HEK cells for gRNA orthogonality experiment

Screen Shot 2016-08-01 at 12.15.02.png

PLATE 1

	1	2	3	4	5	6	7	8
A	pCJH2 T40 BW363 pGOP23	pCJH2 T40 BW363 pGOP25	pCJH2 T40 BW363 pGOP28	pCJH2 T40 BW363 pGOP30	pCJH2 T40 BW363 pGOP35	pCJH2 T40 BW363 pGOP39	pCJH2 T40 pGEX3 pGOP23	pCJH2 T40 pGEX3 pGOP25
B								
C								
D	pCJH2 T40 pGEX3 pGOP28	pCJH2 T40 pGEX3 pGOP30	pCJH2 T40 pGEX3 pGOP35	pCJH2 T40 pGEX3 pGOP39	pCJH2 T40 pGEX5 pGOP23	pCJH2 T40 pGEX5 pGOP25	pCJH2 T40 pGEX5 pGOP28	pCJH2 T40 pGEX5 pGOP30
E								
F								

PLATE 2

	1	2	3	4	5	6	7	8
A	pCJH2 T40 pGEX5 pGOP35	pCJH2 T40 pGEX5 pGOP39	pCJH2 T40 pGEX8 pGOP23	pCJH2 T40 pGEX8 pGOP25	pCJH2 T40 pGEX8 pGOP28	pCJH2 T40 pGEX8 pGOP30	pCJH2 T40 pGEX8 pGOP35	pCJH2 T40 pGEX8 pGOP39
B								
C								
D	pCJH2 T40 pGEX10 pGOP23	pCJH2 T40 pGEX10 pGOP25	pCJH2 T40 pGEX10 pGOP28	pCJH2 T40 pGEX10 pGOP30	pCJH2 T40 pGEX10 pGOP35	pCJH2 T40 pGEX10 pGOP39	pCJH2 T40 pGEX15 pGOP23	pCJH2 T40 pGEX15 pGOP25
E								
F								

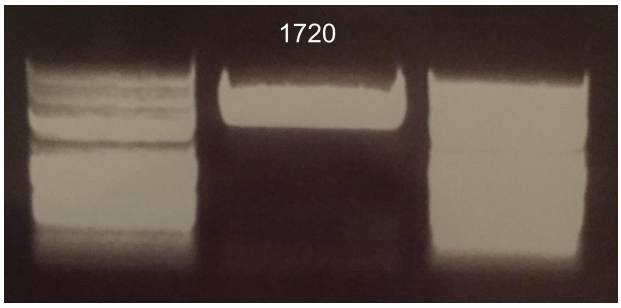
PLATE 3

	1	2	3	4	5	6	7	8
A	pCJH2 T40 pGEX15 pGOP28	pCJH2 T40 pGEX15 pGOP30	pCJH2 T40 pGEX15 pGOP35	pCJH2 T40 pGEX15 pGOP39	pCJH2 T40 pGEX19 pGOP23	pCJH2 T40 pGEX19 pGOP25	pCJH2 T40 pGEX19 pGOP28	pCJH2 T40 pGEX19 pGOP30
B								
C								
D	pCJH2 T40 pGEX19 pGOP35	pCJH2 T40 pGEX19 pGOP39						
E								
F								

WEDNESDAY, 7/6

Miniprep pGEX, GFP pGOP, and mRuby pGOP 6& 14
Digested 1720 with AgeI and EcoRI

07-06-2016 1720.jpeg



Redigested 2485, 2487, and 2489 with NotI and Cip

07-06-2016 2485 2487 2489.jpeg

Ligated and transformed pBEX1-24 pPV1,5-8
Retransformed pGEX6 and 14 pGOP26,34,66,74

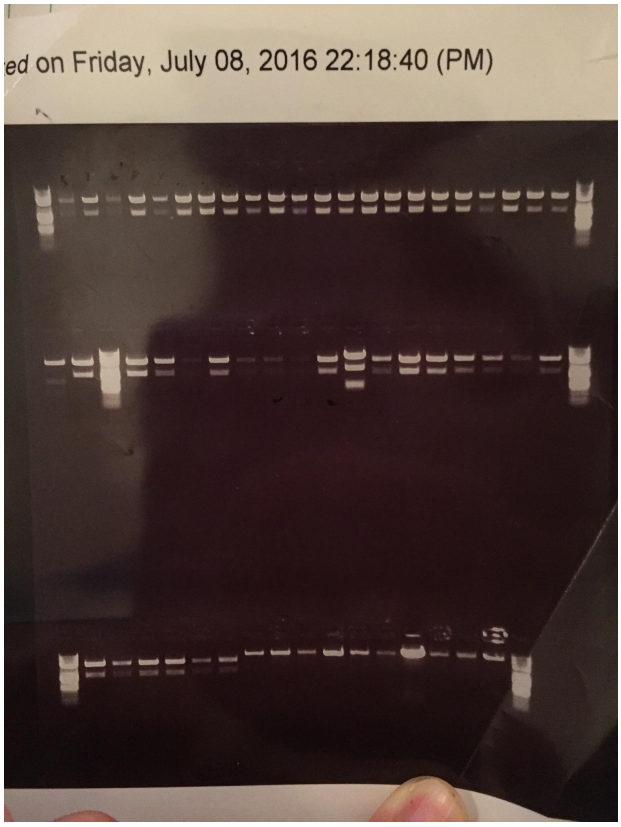
Made LB + Carb plates

THURSDAY, 7/7

Picked colonies for pBEX 1 - 24, pGEX 6 and 14, GFP and mRuby pGOPs 6 and 14, adn pPVs 1, 5, 6, 7, 8
Miniprep pGEX 1-4
Ran FACs
Grew more 2534

FRIDAY, 7/8

IMG_9323 copy.jpg



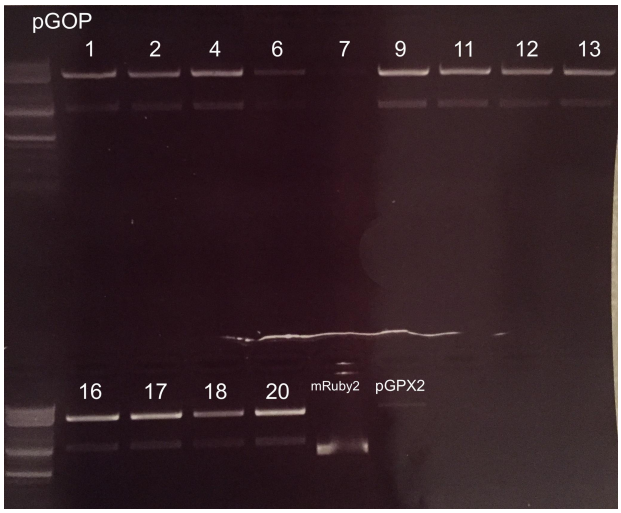
MONDAY, 7/11

Grew up pPV7 and pBEX 10, 12, 13, 17, 18, 22
Growing more 2534
Gibsoned pREC1, 2, and 3
Passaged HEK cells

TUESDAY, 7/12

Cell Stock pPV7 and pBEX 10, 12, 13, 17, 18, and 22
Digested pGPX2, mRuby2, and pGOP 1, 2, 4, 6, 7, 9, 11, 12 13, 16, 17, 18, and 20
    mRuby2 and pGOPs digested with MluI and AgeI
    pGPX2 digested with BbsI
    Ran on a gel and gel extracted

07-12-2016 pGOP mRuby2 pGPX2.jpeg



WEDNESDAY, 7/13

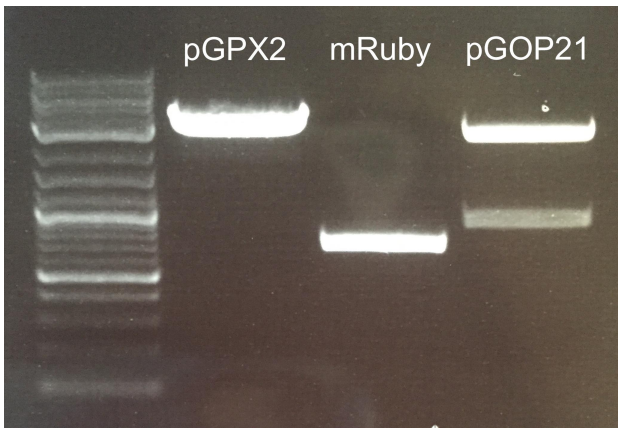
Miniprep pGPX2, 1720, 1942-44

Digested pGPX2, 1720, 1942-44 with _____

Re-digested pGPX2 (bbs1, extract 3kb band) ,mRuby2(mlu1, age1; extract 700bp band) ,and pGOP21 (mlu1,age1, extract 3kb band)

ran on gel and gel extracted

07-13-2016 pGPX2,mRuby,pGOP21.jpeg



THURSDAY, 7/14

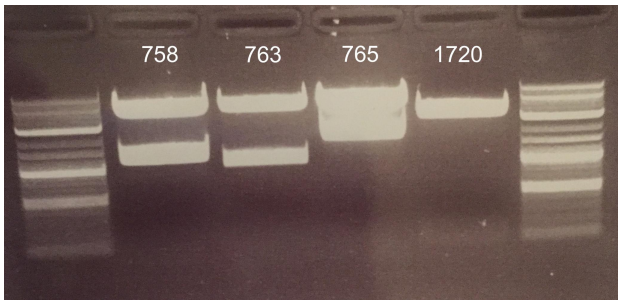
Ligating and transforming

pGOP 21 22 24 27 29 31 32 33 34 36 37 38 40 41 42 44 47 49 51 52 53 56 57 58 60 61 62 64 66 67 69 71 72 73 76 77 78 80

Digesting

1270 758 763 765 with AgeI and EcoRI

07-14-2016 758 763 765 1720.jpeg



Circuit Designs Digesting

- Part1 AscI NotI
- Part2 AscI NotI
- Part3 AscI NotI
- Part4 AscI NheI
- Dest EcoRI NotI

Circuits (P1-P2-P3-P4)

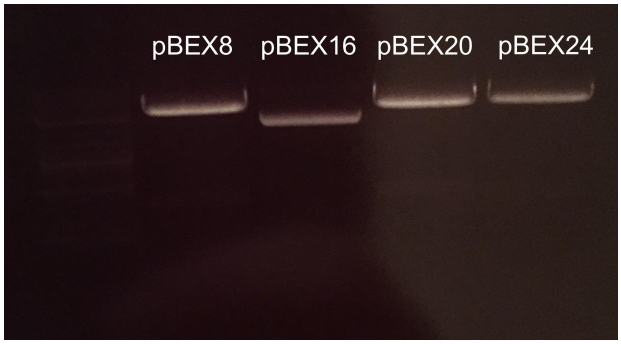
pBEX1-pBEX6-pBEX15-pBEX24

pBEX1-pBEX10-pBEX19-pBEX20

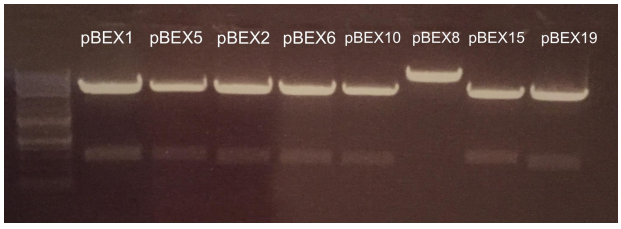
pBEX5-pBEx18-pBEX19-pBEX16

pBEX5-pBEX2-pBEX15-pBEX8

07-14-2016 pBEX 8 16 20 24.jpeg

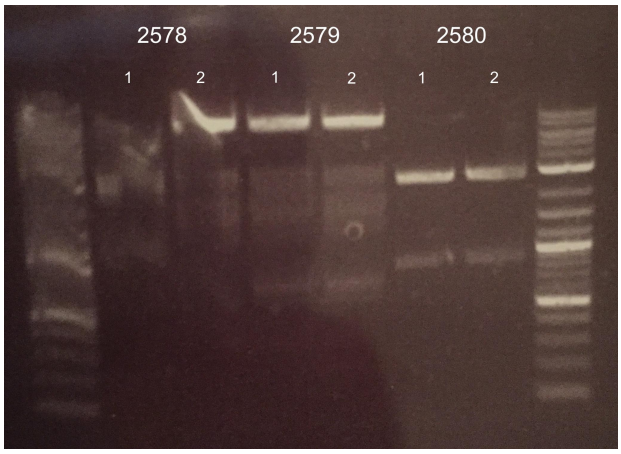


07-14-2016 pBEXs.jpeg



Test cut 2578, 2579, 2580 (formely pRECs)

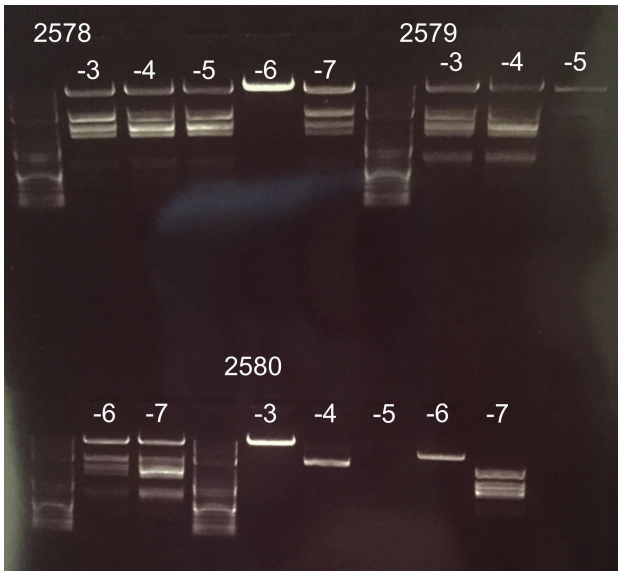
07-14-2016 2578 2589 2580.jpeg



FRIDAY, 7/15

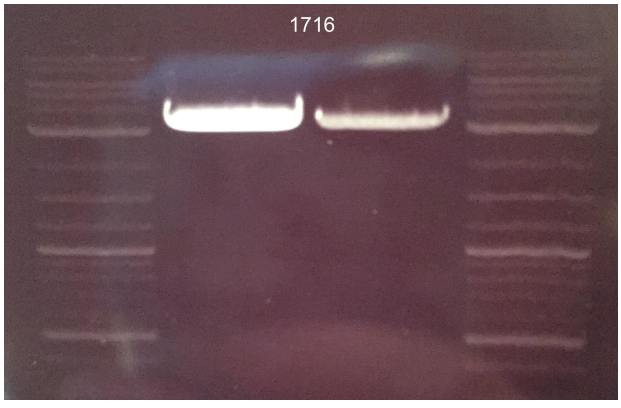
Miniprep 2578, 2579, and 2580 3-7 & testcut

07-15-2016 2578 2579 2580 test cut.jpeg



2578-2 and 2579-1 were sequenced verified adn moved to cell stock
Minipreped 1716 pDest
Digested pDest with EcoRI and NotI

07-15-2016 1716.jpeg



Religated pPV1, pPV6, and pPV8
Picked new pGOP colonies for GFP only

SATURDAY, 7/16

Miniprepped new GFP clonies
Minprepped 2580-8 through 2580-17
Transformed pCir1 pCir2 and pCir3

SUNDAY, 7/17

Test Cuts for 2580 aditional colonies
Test Cuts for pPV1, pPV6, and pPV8 additional colonies
Test Cuts for pGOP GFP additional colonies

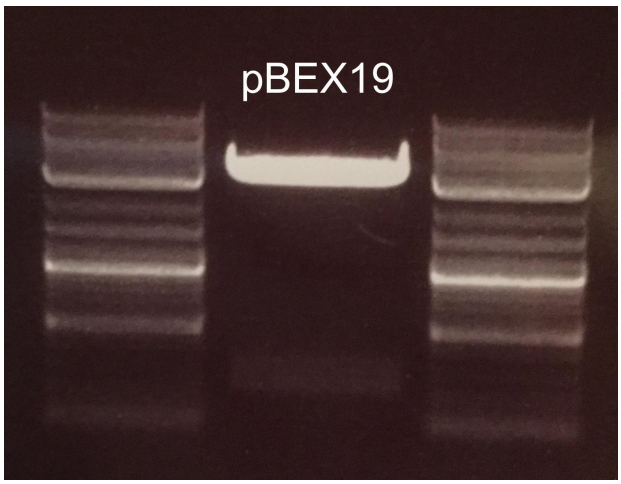
MONDAY, 7/18

Test Cuts for pCIR1, pCir2, and pCir3
Test Cuts for pGOp1 and 2 under BFP and mRuby
Split HEK cells 1:10
Created two 48 well plates at 200,000 cells/ml for transfection of BFP pGOP constructs
Created two T175plates with 25ml of 100,000cells/ml for stable integration

TUESDAY, 7/19

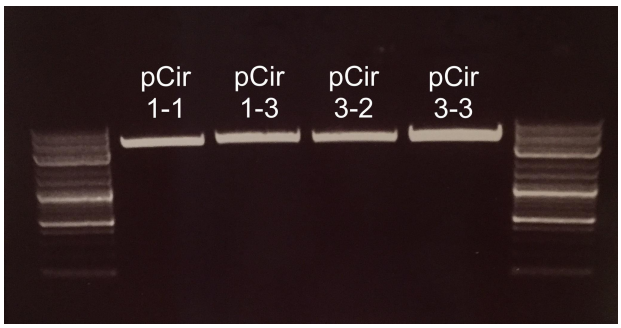
Midiprep BW361, BW465, BW471, BW 474, 2578, and 2579
Digested pBEX 19 with AscI and NotI
Gel extracedracted pBEX 19

07192016 pBEX19.jpeg



Digested pCir 1-3, 1-1, 3-2, 3-3 with Not1

07192016 pcirtestcuts.jpeg



Transfected BFP

Screen Shot 2016-08-01 at 00.01.59.png

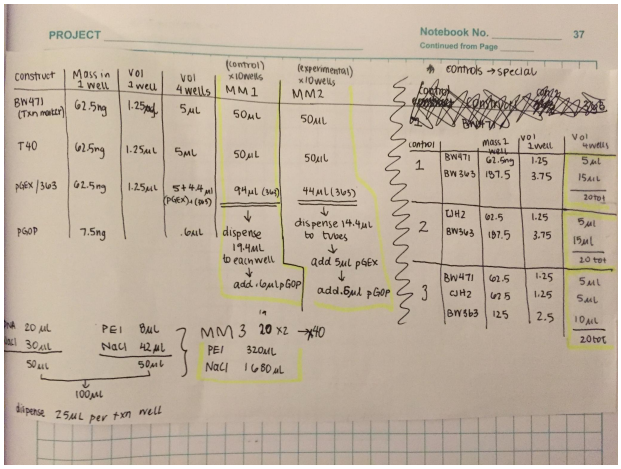
PLATE 1

	1	2	3	4	5	6	7	8
A	BW471 (iRFP) T40 BW363	BW471 T40 BW363 pGOP5	BW471 T40 BW363 pGOP6	BW471 T40 BW363 pGOP8	BW471 T40 BW363 pGOP10	BW471 T40 BW363 pGOP14	BW471 T40 BW363 pGOP15	BW471 T40 BW363 pGOP19
B								
C								
D	BW471 T40 pGEX3 pGOP3	BW471 T40 pGEX5 pGOP5	BW471 T40 pGEX6 pGOP6	BW471 T40 pGEX8 pGOP8	BW471 T40 pGEX10 pGOP10	BW471 T40 pGEX14 pGOP14	BW471 T40 pGEX15 pGOP15	BW471 T40 pGEX19 pGOP19
E								
F								

PLATE 2

	1	2	3	4	5	6	7	8
A	BW471 BW363	CJH2 BW363	BW471 CJH2 BW363	WT blank				
B								
C								
D								
E								
F								

IMG_0128.jpeg



WEDNESDAY, 7/20

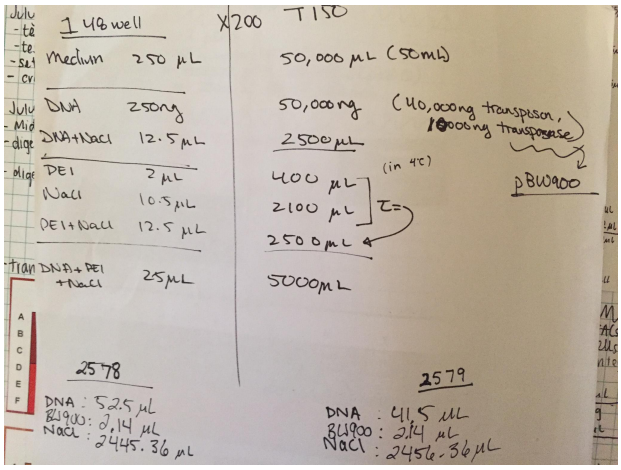
THURSDAY, 7/21

Performed FACs on the BFP transfected cells. Tried to create the data in MEFL but had trouble with getting the channel names to work with the software.

Split HEK cells to P16. Expanded them into large T175 plate

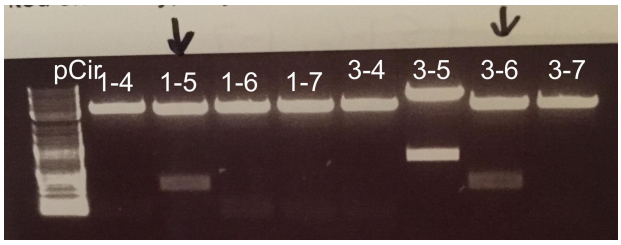
Transfected the HEK cells in T175 with 2578 and 2579. Brought media volume up to 50ml to do so

IMG_1338.jpeg



FRIDAY, 7/22

07222016 pCir test cut2.jpeg



will did a testcut of pCir1 and pCir3. sent one of each in for sequencing.

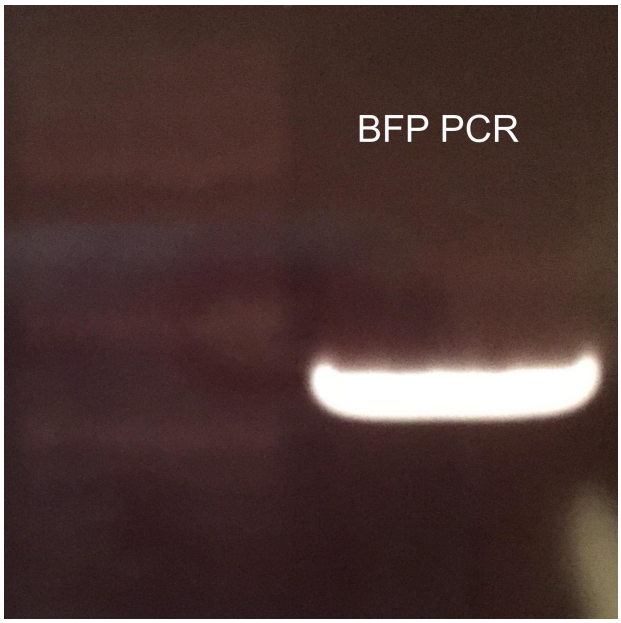
SATURDAY, 7/23

added puromyocin to the two stable line integrations of 2578 and 2579. 2ug per ml so we added 10ul of puromyocin to 50ml of media in the flask

many more cells ttoday than yesterday

Sent in a sample of the pGOP41 used in the previous transfection to troubleshoot what went wrong and why that particular gRNA was not working with BFP

07232016 BFP PCR.jpeg



SUNDAY, 7/24

Split the stable line integration HEK cells of 2578 and 2579 each into two new T175 flasks because they were too crowded. Added puromyocin to the fresh media.

Transformed (GFP) pGOP 26, 27, 32, 33, 38, 40

(BFP): pGOP 41, 42, 43, 44, 46, 47, 49, 51, 52, 53, 56, 57, 58, 60

(mRuby): pGOP 61, 62, 64, ,66, 67, 69, 71, 72 73, 76, 77, 78, 80

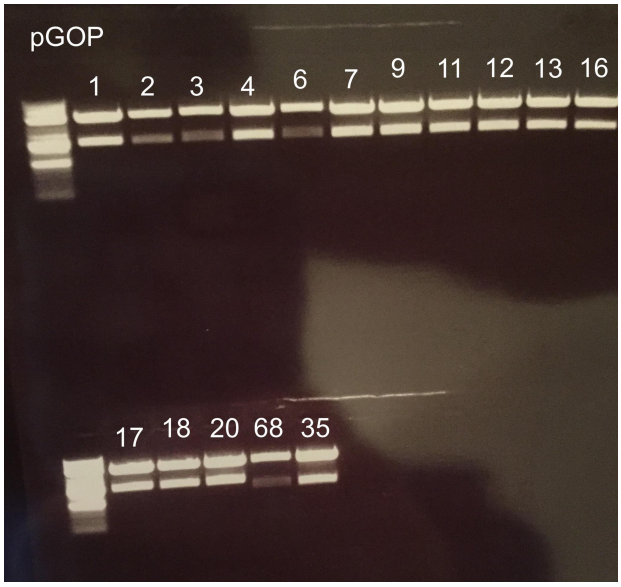
MONDAY, 7/25

PIcked colonies from all transformed GFP pGOPs and less successful BFPs and mRubys

Prepped overnight ligation for the BFPs and mRubys

due to miscommunication, the ligation mix was made incorrectly but we went ahead anyway

07252016 pGOP cuts and BFP mRUBY.jpeg



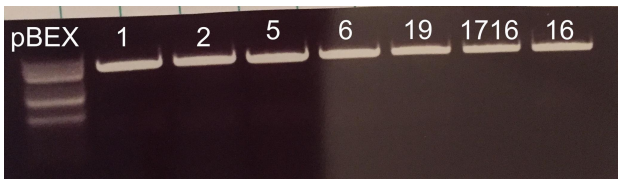
TUESDAY, 7/26

Digested pBEX 1,2,5,6,16,19 (AscI and NotI except 16 which was AscI and NheI) and 1716 (EcoRI and NotI) and pGOP68 and 35 (MluI and AgeI)

did not extract the pGOPs or 1716

extracted the rest

07262016 pBEX cuts.jpeg

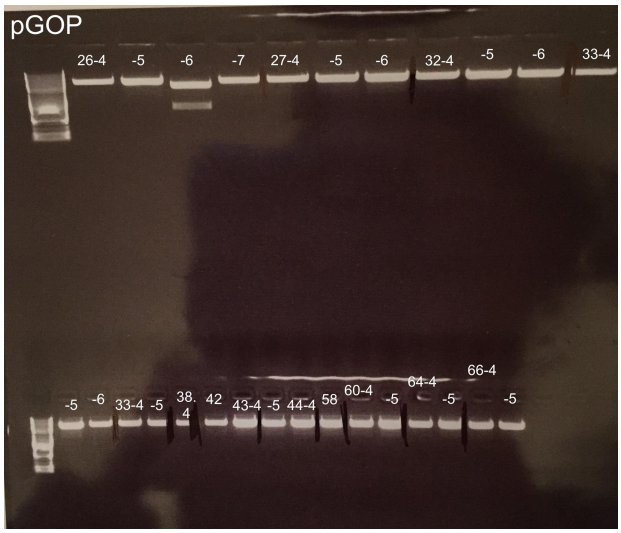


Inoculated pBEX 1,6,16,19, and 1716 and pGPX2 (so we could drop in the new oligos)

Transformed DNA from overnight ligation

made Cam plates

07262016 pgop.jpeg



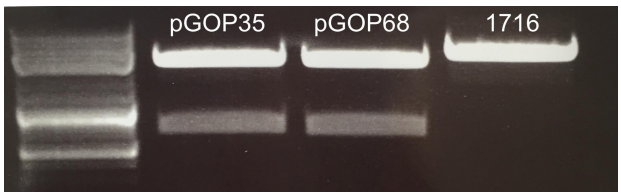
WEDNESDAY, 7/27

Transformation did not work

Digested 1716 (EcoRI and NotI) and pGOP68 and pGOP35 (MluI and AgeI)

gel extracted

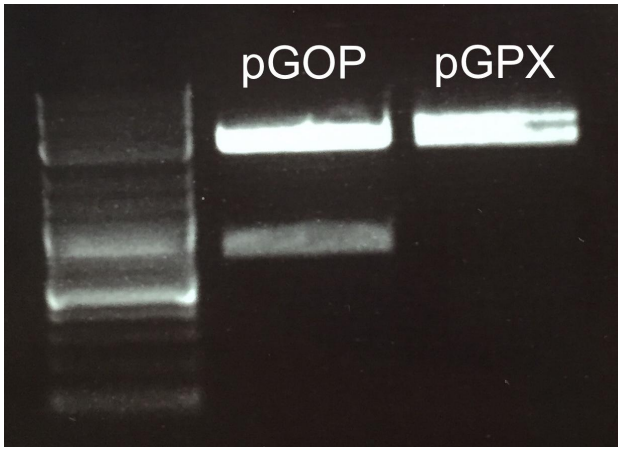
07272016 pGOP35, 68 1716.jpeg



annealed multimerized oligos

digested more pGPX

07272016 pGOP and pGPX digest.jpeg



Diluted all the DNA for the transfection

Transfected HEK cells with the "mini circuit" (1902/1903, pGOP1, pGOP22, BW390 (constituitive cre) and 2196

also transfected pGOP22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 to test all of the GFPs. we did not have pGOP 21 and we did not have pGOP 40. We did not have pGEX11 to transfect so pGOP31 should not work.

organized our DNA boxes

Screen Shot 2016-08-01 at 12.13.53.png

PLATE 1

	1	2	3	4	5	6	7	8
A	CJH2 T40 BW363 BW363 pGOP1 pGOP22	CJH2 T40 BW363 BW390 pGOP1 pGOP22	CJH2 T40 1902 BW363 pGOP1 pGOP22	CJH2 T40 1903 BW363 pGOP1 pGOP22	CJH2 T40 1902 BW390 pGOP1 pGOP22	CJH2 T40 1903 BW390 pGOP1 pGOP22	IRFP T40 1902 BW363 2196	IRFP T40 1903 BW363 2196
B								
C								
D	IRFP T40 1902 BW390 2196	IRFP T40 1903 BW390 2196						
E								
F								

PLATE 2

	1	2	3	4	5	6	7	8
A	CJH2 T40 BW363 pGOP22	CJH2 T40 BW363 pGOP23	CJH2 T40 BW363 pGOP24	CJH2 T40 BW363 pGOP25	CJH2 T40 BW363 pGOP26	CJH2 T40 BW363 pGOP27	CJH2 T40 BW363 pGOP28	CJH2 T40 BW363 pGOP29
B								
C								
D	CJH2 T40 BW363 pGOP30	CJH2 T40 BW363 pGOP31	CJH2 T40 BW363 pGOP32	CJH2 T40 BW363 pGOP33	CJH2 T40 BW363 pGOP34	CJH2 T40 BW363 pGOP35	CJH2 T40 BW363 pGOP36	CJH2 T40 BW363 pGOP37
E								
F								

PLATE 3

	1	2	3	4	5	6	7	8
A	CJH2 T40 BW363 pGOP38	CJH2 T40 BW363 pGOP39	CJH2 T40 pGEX2 pGOP22	CJH2 T40 pGEX3 pGOP23	CJH2 T40 pGEX4 pGOP24	CJH2 T40 pGEX5 pGOP25	CJH2 T40 pGEX6 pGOP26	CJH2 T40 pGEX7 pGOP27
B								
C								
D	CJH2 T40 pGEX8 pGOP28	CJH2 T40 pGEX9 pGOP29	CJH2 T40 pGEX10 pGOP30	CJH2 T40 pGEX11 pGOP31	CJH2 T40 pGEX12 pGOP32	CJH2 T40 pGEX13 pGOP33	CJH2 T40 pGEX14 pGOP34	CJH2 T40 pGEX15 pGOP35
E								
F								

PLATE 4

	1	2	3	4	5	6	7	8
A	CJH2 T40 pGEX16 pGOP36	CJH2 T40 pGEX17 pGOP37	CJH2 T40 pGEX18 pGOP38	CJH2 T40 pGEX19 pGOP39	GFP	BFP	IRFP	GFP BFP
B								
C								
D	GFP IRFP	BFP IRFP	GFP BFP IRFP	WT				
E								
F								

THURSDAY, 7/28

FRIDAY, 7/29

Digested more BFP and mRUBY

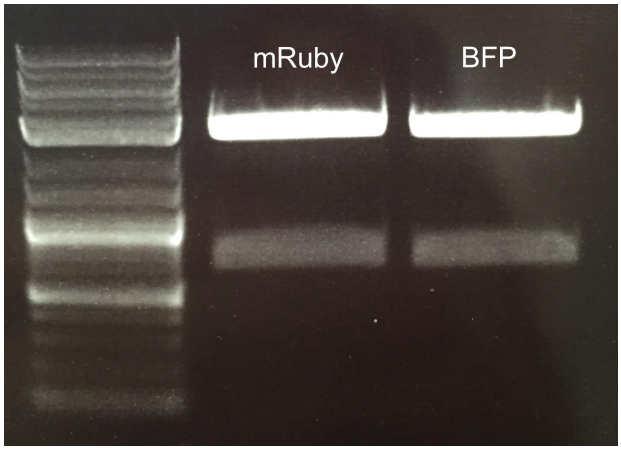
26ul pGOP55 30ulpGOP64

1.5 of MLU1-HF &Age1-HF 1.5 of Mlu1-HF &Age1-HF

5 cutsmart 5 cutsmart

20 h2o 16 h2o

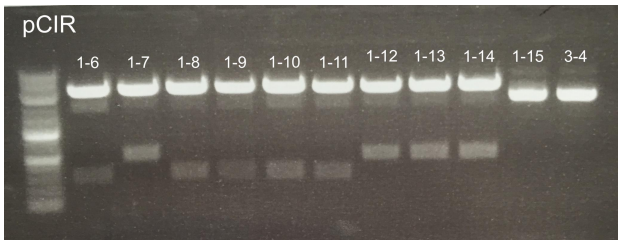
07292016 mRuby BFP.jpeg



ligated to create pGOP41, 51, 82, 83 to se what wasnt working with our cloning by testing our T4 ligase vs Wong labs t4 ligase

Testcut pCir1-6, -7, -8, -9, -10, -11, -12, -13, -14, -15, pCir3-4 with Acs1 Not1-HF

07292016 pCir test cut.jpeg



Cell stocked pGOP38-4, pGOP42-1, pGOp43-1, pGOP44-1, pGOP26-5, pGOP27-4, pGOP32-4, pGOP32-4, pGOP33-4, pGOP60-1, pGOP64-1

performed FACs
Split Deboki's cells for her and took a t175 plate at 1:10. (passage again on monday)
Moved the stable line integrations to T50 plates. added 2ul of puromycin to 10ml of media

Picked colonies for GOP 82, and 41, 51 (no colonies for 83), pCIR1s that Ben made (picked 10 colonies)
Met with Leidy to discuss what we are doing wrong with our ligation and transformation protocol.
Re did digesestion of BFP and mRuby from pGOP 43 and 64, respectively. (age1 mlu1)
Redigested pgpx2 to drop in the oligos.
Religating fluorescence into regular pGOPs

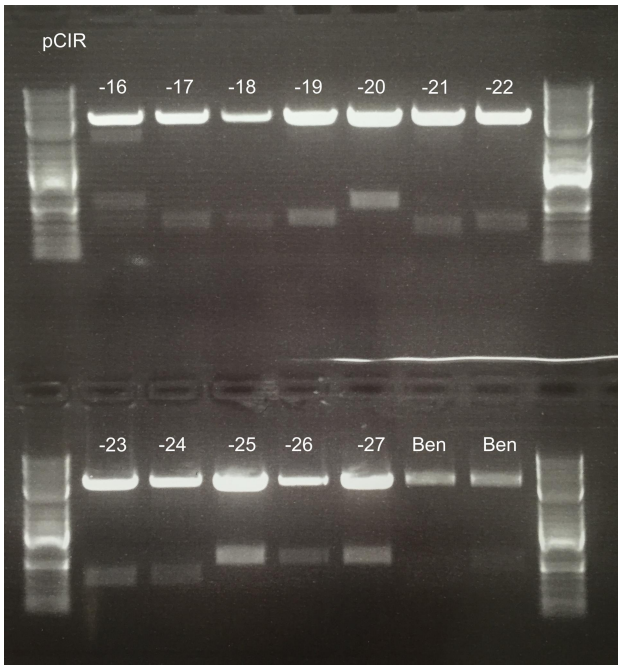
SATURDAY, 7/30

Picked colonies of pGOP 66-1*(failed),-2, 67-1*(failed),-2, 71-1*(failed),-2, 73-1*(failed),-2, 76-1*(worked),-2, 46-1*(worked),-2, 47-1*(failed),-2., 51-1*(failed),-2, 52-1*(failed), 53-1*(worked),-2, 56-1(failed)*,-2, 49-1*(failed),-2, 69-1*(failed sequencing reaction),-2, 41-1*(worked),-2,-3 -4,-5, 51-1,-2,-3,-4,-5,-6, 40-1,-5
pGOP 81-1,-2, 82-1*,-2, 83-1,-2,84-1,-2, 85-1,-2, 82-0* (-0 means grew up day before), 86-1,-2,87-1,-2,88-1*,-2, 89-1,-2, 90-1,-2, 83-0
pCIR1 -16,-17-,-18, -19,-20*,-21,-22,-23, -24,-25*,-26*,-27*, Ben's1, Ben's2*
(starred**** ones sent for sequencing)

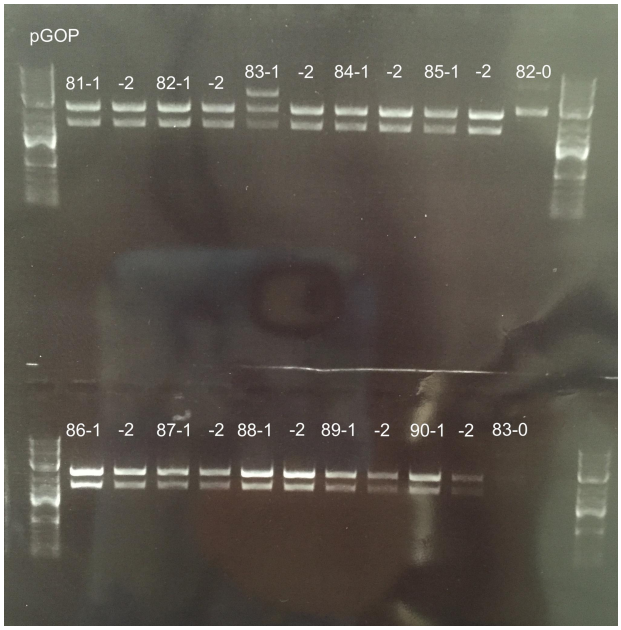
SUNDAY, 7/31

miniprep of all of the colonies picked yesterday
Ran test cuts of all of the colonie picked

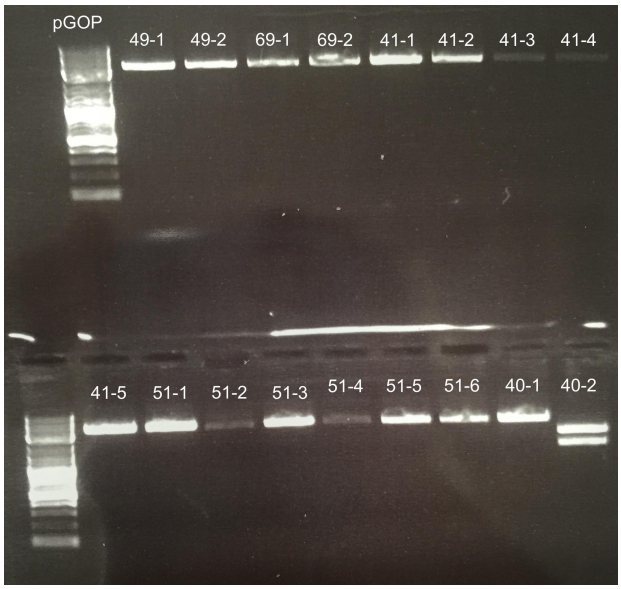
07312016 pCIR test cuts.jpeg



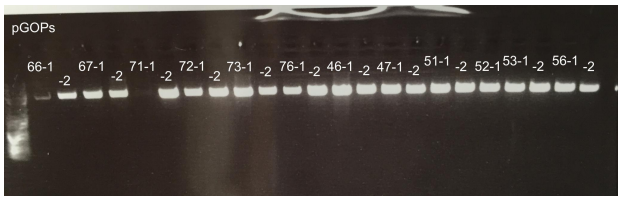
07312016 pGOP80 test cuts.jpeg



07312016 pGOP test cuts more.jpeg



07312016 pGOP cuts again.jpeg



MONDAY, 8/1

split HEK cells 1:10 from large flask into small flask.
let stable line of cells grow for another day.
Grew up.... pGEX11, pGEX12, pGEX13, pGEX17, pGEX18, pGEX20, pGOP6(x2),pGOP21, pGOP22, pGOP23, pGOP24, pGOP25, pGOP26, pGOP27, pGOP28, pGOP29, pGOP30, pGOP31, pGOP32, pGOP33, pGOP34, pGOP35, pGOP36, pGOP37, pGOP38, pGOP39, pGOP42, pGOP43, pGOP44(x2), pGOP45(x2), pGOP50, pGOP54, pGOP55(x2), pGOP59, pGOP68, pGPX2

TUESDAY, 8/2

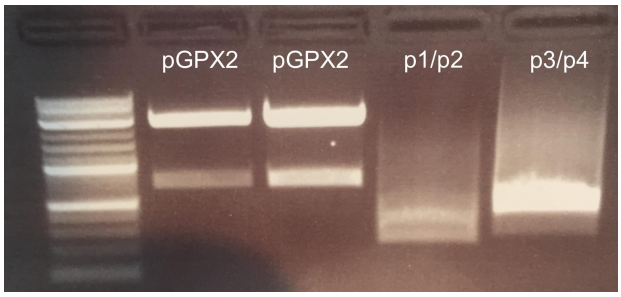
(Will and Marisa) miniprepped pGEX11, pGEX12, pGEX13, pGEX17, pGEX18, pGEX20, pGOP6(x2),pGOP21, pGOP22, pGOP23, pGOP24, pGOP25, pGOP26, pGOP27, pGOP28, pGOP29, pGOP30, pGOP31, pGOP32, pGOP33, pGOP34, pGOP35, pGOP36, pGOP37, pGOP38, pGOP39, pGOP42, pGOP43, pGOP44(x2), pGOP45(x2), pGOP50, pGOP54, pGOP55(x2), pGOP59, pGOP68, pGPX2
Grew up pGOP41, pGOP46, pGOP53, pGOP71, pGOP76 for cell stock
Split the stable line HEK cells into new T75 flasks (p18)

WEDNESDAY, 8/3

suspended the crRNA gBlocks oligos in 1xTE

Digested pGPX2 with BBs1

20160803 jeffery's stuff.jpeg



DIgested pGOP76-1 and pGOP 41-1 for mRuby and BFP
Transformed pGOPs 57, 58, 61, 62, 66, 77, 78, 81, 84, 85, 86, 87, 88, 89 using Leidys transformation protocol
- use Ben's Top Ten cells, let cells sit on ice
- add 10ul of 5x KCM to ligation mix
- add 30ul of DI H2O to ligation mix
- add all 50 ul to cells
- plated 95ul of cells onto 1/2 plate

Sent sequencing pGOP 47-1(worked), 49-1(worked), 51-2(worked), 52-1(worked), 56-2 (failed), 67-1(worked), 69-1(worked), 71-1(FAIL), 72-1(worked), 73-1(resendfor seuquencing), 83-0(resend for sequencnig)

Cell stocked pGOP41, pGOP46, pGOP53(need to throw awayb/c miscommunication), pGOP76 (throw away b/c miscommunication)

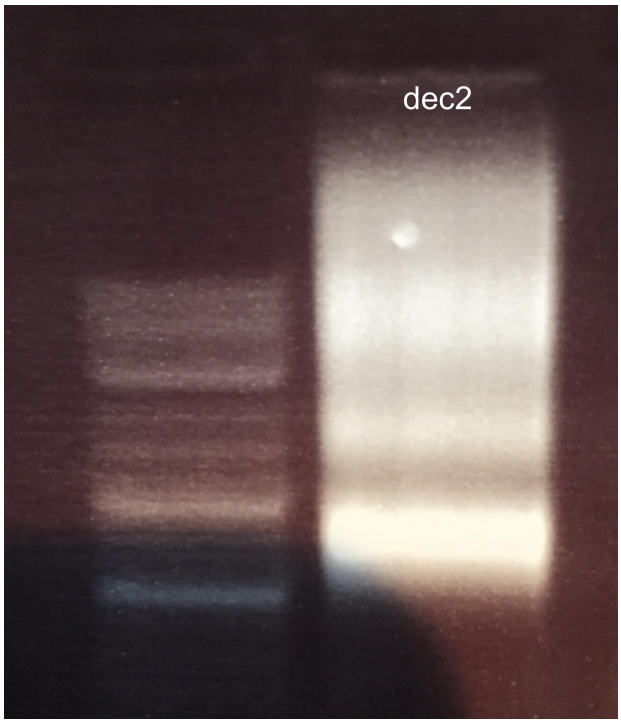
THURSDAY, 8/4

(Marisa) Picked 2 colonies for pGOPs 57, 58, 61, 62, 66, 77, 78, 81, 84, 85, 86, 87, 88, 89

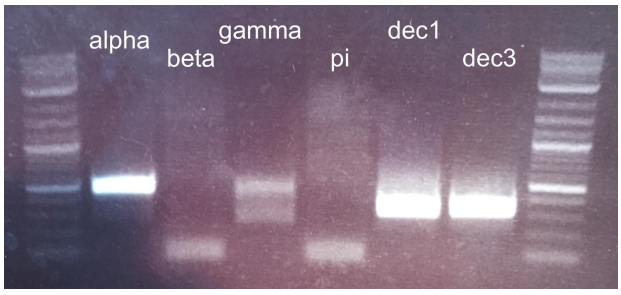
(Kami) Split wildtype cells 1:20 to P19

(Jeffery) dimerized guides 1, 3, 8, 13 in various configurations with each other (1-3, 3-1, 8-13, 13-8)
- this involved PCR and a gel extraction
- Designed decoders

20160805 dec2.jpeg



20160805 decoder parts.jpeg



(Will) Grew up pGOP 47, 49, 51, and 82-0 for cell stock

Due to a miscommunication error, we had to retransform and plate pGOP52-1, pGOP53-1, pGOP67-1, pGOP69-1, pGOP71-2, pGOP72-1, pGOP73-1. We did this with our typical transformation protocol.

FRIDAY, 8/5

(Marisa and Will) Miniprepeed pGOPs 57, 58, 61, 62, 66, 77, 78, 81, 85, 86, 87, 88, and 89

Due to a miscommunication error, miniprepped pGOP 47, 49, 51, and 82-0

(Will) Gibsoned

pCir 2 using 1716, A, B, C, D

pCir 2 using 1716 Part1/2, Part 3/4

pCir 2 usng 1716, Decoder 2

pCir 4 using 1716, lambda, Part 3/4

pCir 1 using 1716, alpha, Bex19

pCir 1 using 1716, Part 1/2, Bex19, Bex16

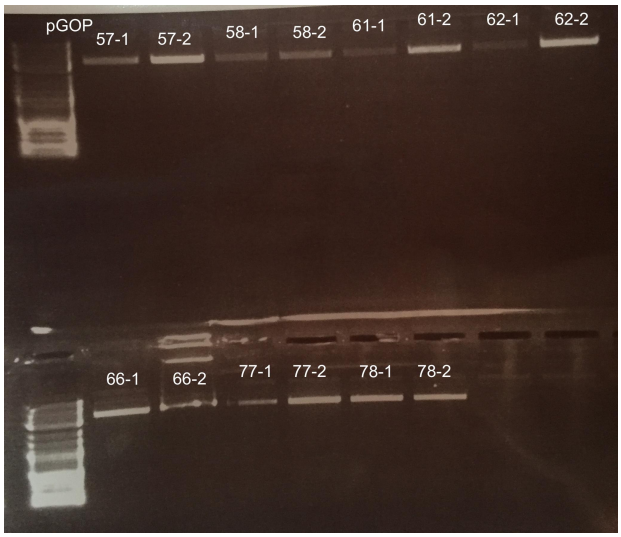
pCir 1 using 11716 Bex 1, Bex6, Bex19, Bex16

pCir 3 using 1716, Bex5, Bex2, Bex19, Bex16

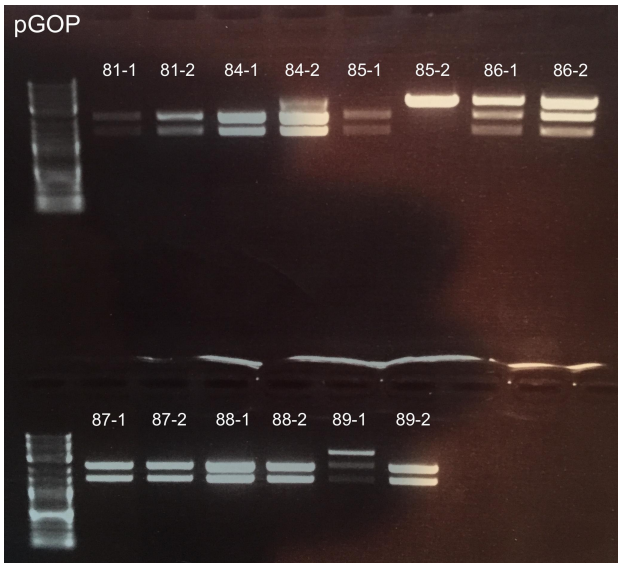
(Kami) Split stabel line (2578 and 2579) 1:10 in T75

(Rachel) Test-cut pGOPs 57, 58, 61, 62, 66, 77, 78, 81, 85, 86, 87, 88, and 89

20160805 pGOP57-78 test cuts.jpeg



20160805 pGOP81-89 test cut.jpeg



SATURDAY, 8/6

(Rachel) digested pGPX2 (5mg) with 3ul of BbsI. Double digested - too low of concentraiton to proceed with ligation

SUNDAY, 8/7

(Will) grew up

MONDAY, 8/8

(Jeffery) Digested pgpx2 with bbsi
(kami) made WT Hek plates with Yash and split WT and 2578 and 2579 integrated cells 1:10
(will) cell stocked pGPX2
(Will) Test cut the pCir gibsons with Asc1 and Not1
(Will) transformed pGOP 84. 86, 87, 88, 89, 90 with a ligation using Leidy's protocol
transformed pgOP47, 49, 51, 82-0 without a ligation using Leidy's protocol

TUESDAY, 8/9

(Rachel) Growing pCir4_2_4 from stab plate as sequencing indicated it worked!
(Rachel) Picked colonies from pGOP 81,84,86,87,88,89,90
(Rachel) made more LB + Cam broth
(Kami) autoclaved tips and agar
(Marisa and Kami) made carb plates
(Jeffrey) performed overhang extension PCRs on G-blocks (that make decoder) to build Circuit 2

WEDNESDAY, 8/10

(Marisa and Will) miniprep pGOP 81,84,86,87,88,89,90
test cut with speI and BbsI
(Marisa) Cell Stock pGOP 47, 49, 51, and 82; pCir 4
miniprep pCir 4
(Jeffrey) Digested Bex 19 and 16

THURSDAY, 8/11

(Kami and Marisa) grew up BW 363, 390, 391; pSB1C3; and T40 for midiprep
(Kami) split WT, 2578, 2579 HEK cells into T175
(Jeffrey) Picked Colonies for 87 and 88

FRIDAY, 8/12

(Marisa) Midiprep BW 363, 390, 391, T40, pSB1C3
(Jeffrey) MIinprepped and test 87 and 88... picked colonies for 90. Transformed interlab study plasmids

SATURDAY, 8/13

(Marisa) Midiprep BW 363, 390, 391, T40, pSB1C3
    Cell stock pGOP 87
(Marisa and Rachel) Gel extracted
(Jeffrey) Mini prep and test cut 90... Retransformed interlab device 1

SUNDAY, 8/14

(Marisa and Rachel) Cell stock Interlab study: Pos 1, Neg 1, Part 2-1, Part 2-2, Pos 2, Neg 2, Part 3-1, Part 3-2
    Miniprep Interlab Study and pGOP 56-3/4/5, pGOP 83-5/6/7/8/9
(Rachel) Digested pGOPs 56 and 83 with SpeI and XmaI
(Marisa) Test Cut pGOPs 56 and 83
(Rachel) Ligate

MONDAY, 8/15

(Jeffrey and Marisa) picked and grew up colonies: pGOP 57-1, 61-1/2/3, 62-1/2/3, 66-1/2/3, 77-1/2/3, 78-1/2/3, 80-1/2/3, 88-1/2/3/4/5, 90-1/2/3/4/5
(Kami and Marisa) Diluted and nanodropped
(Kami) made 6 plates for transfection. 3 with WT, 2 with stable line integrations of 2578 and 2579 each.

TUESDAY, 8/16

(Marisa) miniprepped pGOP 57-1, 61-1/2/3, 62-1/2/3, 66-1/2/3, 77-1, 78-1/2/3, 80-1/2/3, 88-1/2/3/4/5, 90-1/2/3/4/5
(Will) Digested Marisa's minipreps
(Kami and Rachel) 7:30 pm transfected HEK cells to test the multimerization and the circuit
    for the circuit. Transfected 1/8 of 250ng of each component. (2 pgops made up 1/8 of the ng transfection and the extra was filled with Luis's pSB2C3 blank)
(Kami And rachel) 2 hours after transfection, we added 4OHT/Absistic acid/Rapalog to appropriate wells

WEDNESDAY, 8/17

THURSDAY, 8/18

(Kami and Rachel) prepared the transfected cells for FACS, and ran facs
(Kami) Cells stocked stabel lines of HEK 2578(m150) and 2579 (m151)
    trypsonize cel
    neutralize with media
    spin cells down
    remove media and break up pellet.
    resuspend pellet in 90%FBS 10%DMSO (1ml per cryotube)
    one T75 makess 2-3 cell stocks
(Marisa) Made LB Broth
    inoculated T40 and BW363 in falcon tubes
    turn LB Broth into LB + Carb broth

FRIDAY, 8/19

(Kami) made cam agar and broth
    miniprep pGOP 2, 6, 83-11/12/13/14/15
(Marisa) made Cam plates
    Cell stocked pGOP 57, 61, 80, 88, and 90
    inoculated 1mL of each T40 and BW363 sampled into flasks with 150 mL LB + Carb
    Digested pGOPs 83 with SpeI and BbsI for test cut

SATURDAY, 8/20

(Marisa) Midiprep three of both T40 and BW363

MONDAY, 8/22

(Marisa) Grew up pGOP 82

(Jeffrey) Grew up pGOP 83-14

(Jeffrey) took OD 600 of the interlab and diluted cells down to .02

(Will) PCR'ed the pCMV fro the iGEM registry

(Will) Gibsoned and transformed the CMV PCR product into pGEX

(Marisa) made 96 well plate for InterLab Study

250 uL DPBS and 2uL cells per sample

(Kami Rachel) prepared HEK plates for transfection at 200,000 cells

TUESDAY, 8/23

(Marisa) Cell stock pGOP 83

Grew up BW 1945, 1946, 1947, and 1948

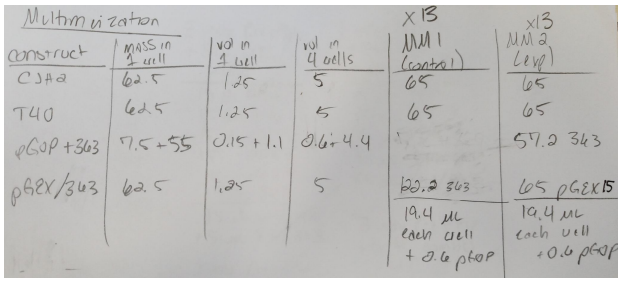
(Will) Miniprep pGOP 82 ad 83

(Jeffrey) Picked colonies from GFP and BFP under igem CMV

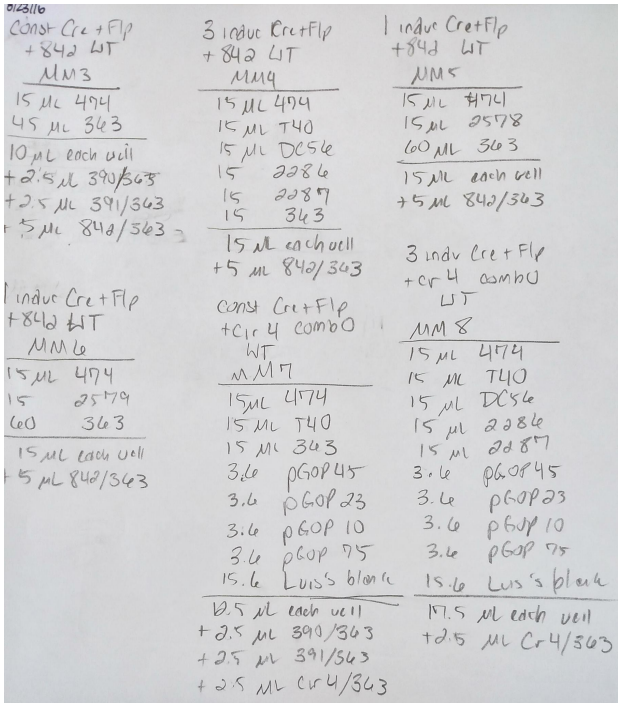
(Jeffrey) Transformed and plated interlab bacteria

(Kami Rachel Marisa) made trasfection DNA mixes and transfected the HEK cells we plated yesterday

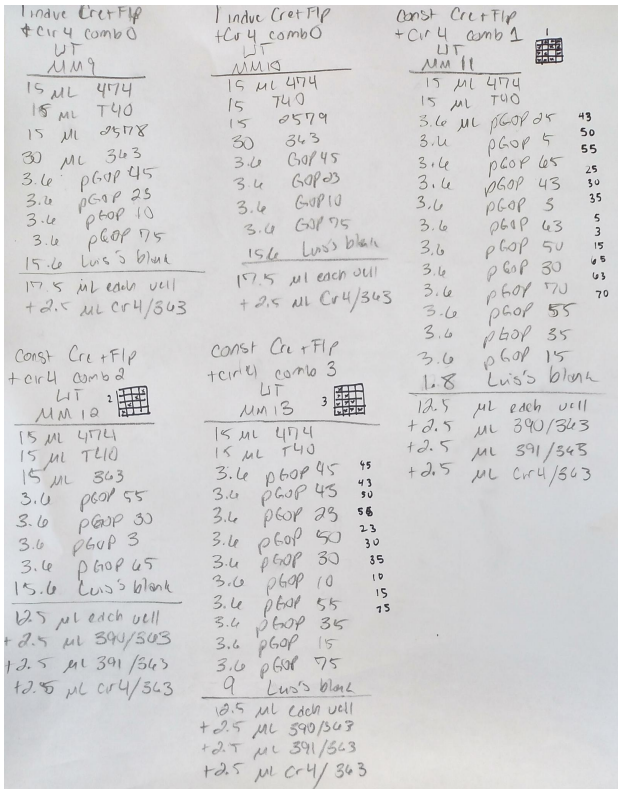
IMG_20160823_145414238.jpg



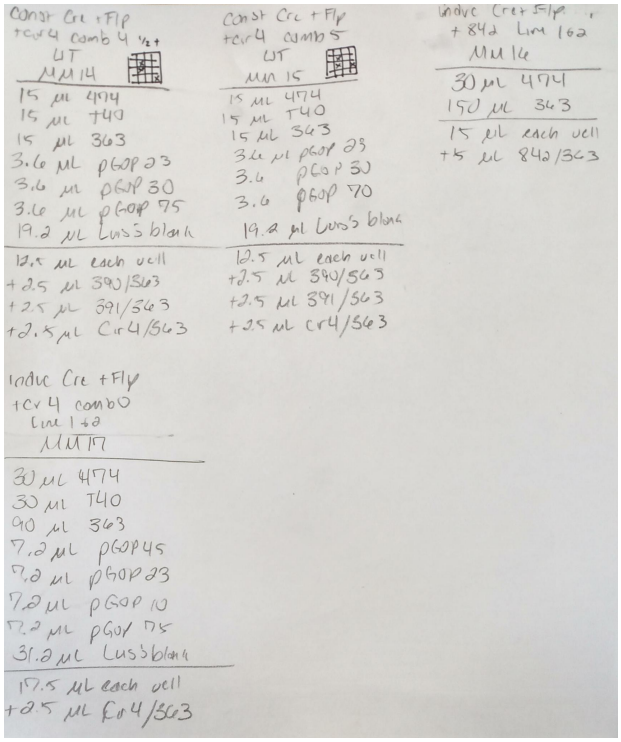
IMG_20160823_145437000_HDR.jpg



IMG_20160823_150756390_HDR.jpg



IMG_20160823_151151878_HDR.jpg



Screen Shot 2016-10-10 at 11.39.05.png

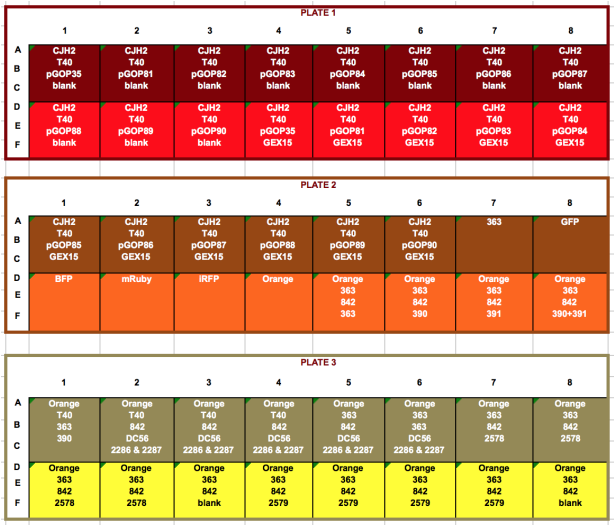


PLATE 1

	1	2	3	4	5	6	7	8
A	CJH2 T40 pGOP35 blank	CJH2 T40 pGOP81 blank	CJH2 T40 pGOP82 blank	CJH2 T40 pGOP83 blank	CJH2 T40 pGOP84 blank	CJH2 T40 pGOP85 blank	CJH2 T40 pGOP86 blank	CJH2 T40 pGOP87 blank
B								
C								
D	CJH2 T40 pGOP88 blank	CJH2 T40 pGOP89 blank	CJH2 T40 pGOP90 blank	CJH2 T40 pGOP35 GEX15	CJH2 T40 pGOP81 GEX15	CJH2 T40 pGOP82 GEX15	CJH2 T40 pGOP83 GEX15	CJH2 T40 pGOP84 GEX15
E								
F								

PLATE 2

	1	2	3	4	5	6	7	8
A	CJH2 T40 pGOP85 GEX15	CJH2 T40 pGOP86 GEX15	CJH2 T40 pGOP87 GEX15	CJH2 T40 pGOP88 GEX15	CJH2 T40 pGOP89 GEX15	CJH2 T40 pGOP90 GEX15	363	GFP
B								
C								
D	BFP	mRuby	iRFP	Orange	Orange 363 842 363	Orange 363 842 390	Orange 363 842 391	Orange 363 842 390+391
E								
F								

PLATE 3

	1	2	3	4	5	6	7	8
A	Orange T40 363 390	Orange T40 842 DC56 2286 & 2287	Orange T40 842 DC56 2286 & 2287	Orange T40 842 DC56 2286 & 2287	Orange T40 842 DC56 2286 & 2287	Orange T40 363 363 DC56 2286 & 2287	Orange 363 842 2578	Orange 363 842 2578
B								
C								
D	Orange 363 842 2578	Orange 363 842 2578	Orange 363 842 blank	Orange 363 842 2579	Orange 363 842 2579	Orange 363 842 2579	Orange 363 842 2579	Orange 363 842 blank
E								
F								

Screen Shot 2016-10-10 at 11.39.27.png

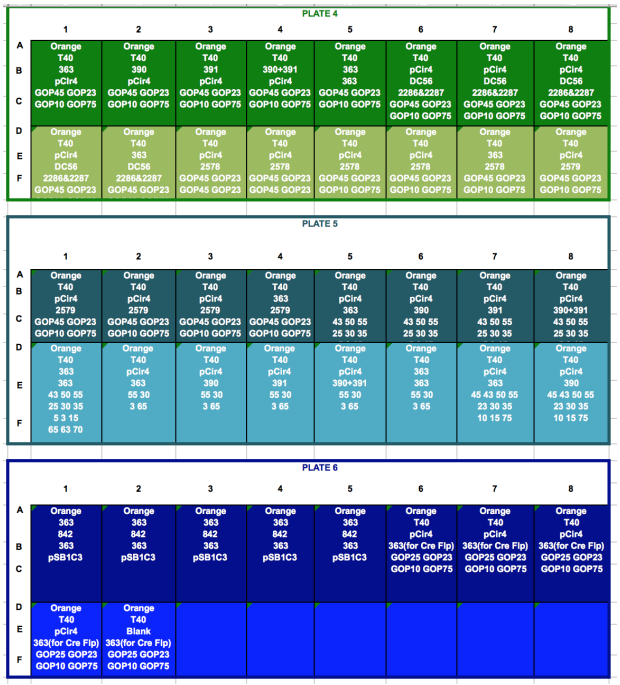


PLATE 4

	1	2	3	4	5	6	7	8
A	Orange T40 363 pCir4 GOP45 GOP23 GOP10 GOP75	Orange T40 390 pCir4 GOP45 GOP23 GOP10 GOP75	Orange T40 391 pCir4 GOP45 GOP23 GOP10 GOP75	Orange T40 390+391 pCir4 GOP45 GOP23 GOP10 GOP75	Orange T40 363 363 GOP45 GOP23 GOP10 GOP75	Orange T40 pCir4 DC56 2286&2287 GOP45 GOP23 GOP10 GOP75	Orange T40 pCir4 DC56 2286&2287 GOP45 GOP23 GOP10 GOP75	Orange T40 pCir4 DC56 2286&2287 GOP45 GOP23 GOP10 GOP75
B								
C								
D	Orange T40 pCir4 DC56 2286&2287 GOP45 GOP23	Orange T40 363 DC56 2286&2287 GOP45 GOP23	Orange T40 pCir4 2578 GOP45 GOP23 GOP45 GOP23	Orange T40 pCir4 2578 GOP45 GOP23 GOP45 GOP23	Orange T40 pCir4 2578 GOP45 GOP23 GOP10 GOP75	Orange T40 pCir4 2578 GOP45 GOP23 GOP10 GOP75	Orange T40 363 2578 GOP45 GOP23 GOP10 GOP75	Orange T40 pCir4 2579 GOP45 GOP23 GOP10 GOP75
E								
F								

PLATE 5

	1	2	3	4	5	6	7	8
A	Orange T40 pCir4 2579 GOP45 GOP23 GOP10 GOP75	Orange T40 pCir4 2579 GOP45 GOP23 GOP10 GOP75	Orange T40 pCir4 2579 GOP45 GOP23 GOP10 GOP75	Orange T40 363 2579 GOP45 GOP23 GOP10 GOP75	Orange T40 pCir4 363 43 50 55 25 30 35	Orange T40 pCir4 390 43 50 55 25 30 35	Orange T40 pCir4 391 43 50 55 25 30 35	Orange T40 pCir4 390+391 43 50 55 25 30 35
B								
C								
D	Orange T40 363 363 43 50 55 25 30 35 5 3 15 65 63 70	Orange T40 pCir4 390 55 30 3 65	Orange T40 pCir4 390 55 30 3 65	Orange T40 pCir4 391 55 30 3 65	Orange T40 pCir4 390+391 55 30 3 65	Orange T40 363 363 55 30 3 65	Orange T40 pCir4 363 45 43 50 55 23 30 35 10 15 75	Orange T40 pCir4 390 45 43 50 55 23 30 35 10 15 75
E								
F								

PLATE 6

	1	2	3	4	5	6	7	8
A	Orange 363 842 363 pSB1C3	Orange 363 842 363 pSB1C3	Orange 363 842 363 pSB1C3	Orange 363 842 363 pSB1C3	Orange 363 842 363 pSB1C3	Orange T40 pCir4 363(for Cre Flp) GOP25 GOP23 GOP10 GOP75	Orange T40 pCir4 363(for Cre Flp) GOP25 GOP23 GOP10 GOP75	Orange T40 pCir4 363(for Cre Flp) GOP25 GOP23 GOP10 GOP75
B								
C								
D	Orange T40 pCir4 363(for Cre Flp) GOP25 GOP23 GOP10 GOP75	Orange T40 Blank 363(for Cre Flp) GOP25 GOP23 GOP10 GOP75						
E								
F								

Screen Shot 2016-10-10 at 11.39.37.png

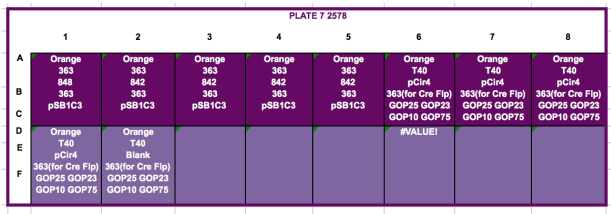


PLATE 7 2578

	1	2	3	4	5	6	7	8
A	Orange 363 848 363 pSB1C3	Orange 363 842 363 pSB1C3	Orange 363 842 363 pSB1C3	Orange 363 842 363 pSB1C3	Orange 363 842 363 pSB1C3	Orange T40 pCir4 363(for Cre Flp) GOP25 GOP23 GOP10 GOP75	Orange T40 pCir4 363(for Cre Flp) GOP25 GOP23 GOP10 GOP75	Orange T40 pCir4 363(for Cre Flp) GOP25 GOP23 GOP10 GOP75
B								
C								
D	Orange T40 pCir4 363(for Cre Flp) GOP25 GOP23 GOP10 GOP75	Orange T40 Blank 363(for Cre Flp) GOP25 GOP23 GOP10 GOP75				#VALUE!		
E								
F								

Added drugs to inducible cells 2hrs after transfection

Drugs are located in pink box labeled "Small molecules"

Rapalog= AC heterodimerizer (1000x)

Absistic Acid (1000x)- vortex before use to get rid of percipitant

4OHT (1000x)

WEDNESDAY, 8/24

(Marisa) Miniprepped and nanodropped pCIR 4, pBex 16 pBEX 19, CMV BFP-1, CMV GFP-1/2, 1945, 1946, 1947, 1948
Grew up Bex 16 and Bex 19
(Jeffrey) Digested 1945, 1946, 1947, and 1948 with BbsI
Grew up pPV 1/5/6/7/8, 1942, 1943, 1944, and 1716
(Marisa) Grew up pCir 4 (x2), DC 56, BW 390, and BW 391
Made LB + Carb

THURSDAY, 8/25

(Rachel and Kami) Ran FACS
(Kami) split HEK293 and stable line cells into big flasks
(Marisa) miniprep pPV 1, 5, 6, 7, 8, pBEX16, 19(x3), BW1942, BW1943, BW1944, BW1716
midipreped DC56, BW390, BW391, pCir4(x2)
(Will) cut for gel extraction 1916, 1945, 1947, 1948 with BBs1
gel extracted those as well
(Will) grew up iGEM CMV for cell stock
(Rachel) annealed the mutated oligos

FRIDAY, 8/26

(Jeffrey and Marisa) Midiprep BW 2286, BW 2287, and pCir 4 (x2)
Cell stock CMV GFP-1
(Rachel) picked two colonies for pGOP 101-150
(Will) Gel extracted pBEX 16 and 19
pBEX 16 digested with AscI and NheI
pBEX 19 digested with AscI and NotI
PCR overhang extension with sequences 37 and 56

SATURDAY, 8/27

(Kami, WIll, Rachel) Performed minipreps for Mismatches and additional multimerized operators pGOP 101-150

MONDAY, 8/29

(Will) Test Cut for Mismatches
(Jeffrey) Attempted overhang extension PCR ... Failed

TUESDAY, 8/30

(Jeffrey) Attempted overhang extension PCR ... Failed
(Jeffrey) Completed Interlab Study
(Marisa) PCR GEX plasmids for CAM transfer
(Will) Gel extracted PCR

WEDNESDAY, 8/31

(Marisa) Digested pGOP 109, 110, 112, 114, 134,l 135, 136, 138
(Marisa) Grew up pGEX 7, pGEX 8, pGPX1,
(Jeffrey) Attempted overhang extension PCR ... Failed
(Jeffrey) Test Cut 109, 110, 112, 114, 134,l 135, 136, 138

THURSDAY, 9/1

(will) pcr mruby and bfp to drop into pgops
(Jeffrey) MInipreped pgop 80-90 for multiplecolors , bexs for circuits, gexs f
(Marisa) Passaged HEK cells
Made 2L of LB Broth, 2L of LB+Carb broth, and 2L of LB + Cam agar for plates
Ligated and transformed pGEX 3, 5, 8, 10, 15, and 19 into Cam (with BW 1721 -n330 backbone)
Grew up T4o (x2), BW 363, GEX 5, GEX 15, pCir 4 (x2), and GPX 2
(Rachel) autoclaved epi tubes and tips
Ligated and transformed BW 1945-1949

FRIDAY, 9/2

(Marisa) Made LB + Cam plates
(Jeffrey) Resuspended cells grown for midiprop and stored in the cold room
(Rachel) Prepared and ran cells for FACS
(Will) Annealed oligos for multimerized pGOP 3

SATURDAY, 9/3

(Jeffrey) transformed mismatched pGOP 101-150
(Marisa and Jeffrey) grew up GEX 3, 5, 8, 10, 15, 19, BW 1721, 1945, 1946, 1947, 1948, and GOP 29

SUNDAY, 9/4

(Jeffrey) picked and grew up colonies from pGOP101-150
    miniprep of GEX 3, 5, 8, 10, 15, 19, BW 1721, 1945, 1946, 1947, 1948, and GOP 29
    Digested BW1945, 1946, 1947, 1948
(Marisa) Cell stock GOP 110, 112, and 114
    Digested GOP 29(MOP) with EcoRI and PstI
    PCR GEX 3, 5, 8, 10, 15, 19 with PrA6 and PrA6r
    PCR clean up BW 1945, 1946, 1947, 1948 and MOP
    Made and ran gel for GEX 3, 5, 8, 10, 15, 19 and BW1721
    (with Kami) gel extracted GEX 3, 5, 8, 10, 15, 19, and BW 1721
    Digested GEX 3, 5, 8, 10, 15, 19 and BW 1721 with EcoRI and PstI
    PCR clean up GEX 3, 5, 8, 10, 15, 19, and BW 1721

MONDAY, 9/5

(Marisa) Cell stock GOP 101-103; 105-108; 111; 115-134; and 146
    Miniprep pGOP 101-103; 105;108; 111; and 115-122
    Ligate and transformed pGEX 3, 5, 8, 10, 15, and 19 into pSB1C3 backbone
(Will) Miniprep pGOP 123-134 and 146
(Kami Rachel) seeded 8 48 well plates at 100,000cells for transfection on wednesday

TUESDAY, 9/6

(Marisa) picked two colonies of GEX 3, 5, 8, 10, 15, and 19 (now termed 103, 105, 108, 110, 115, and 119) from transformation 9/5
(Jeffrey) Performed overhang extension pcr to build the decoder for circuit 2
(Jeffrey) transformed the 18T entry vectors for circuit 5

WEDNESDAY, 9/7

(Marisa) miniprep GEXs 103, 105, 108, 110, 115, and 119
    Test cut with AseI
    picked two colonies for 18T-terminator circuits
(Rachel, Marisa and Kami) transfected HEKcells

THURSDAY, 9/8

(kami Marisa) passaged HEK cells
(jeffrey) performed a gibson to build circuit 2
(jeffrey) test cuts for 18T entry vecotrs for circuit 5 indicated low digestion efficeincy and the circuits needs to be retransformed

FRIDAY 9/9
(Will) Ligated and transformed GEX 21-40 and GOP 151-168
(Will and Marisa) Grew up 3 colonies for GEX 21-40 and GOP 151-168

Saturday 9/10
(Will Jeffrey Marisa) Miniprepped GEX 21-40 and GOP 151-168
(Marisa) PCR GEX 3, 5, 8, 10, 15, 19, and BW 1721 with PrA6 and PrA6r (previous GEX 103, 105 108, 110, 115, 119 were not successful)
(Marisa and Will) gel extracted GEX 3, 5, 8, 10, 15, and 19 and BW 1721 (~400); will be insert for GEX 103, 105, 108, 110, 115, and 119

SUNDAY, 9/11

(Marisa) Digested GEX 103, 105, 108, 110, 115, and 119 and BW 1721 inserts with EcoRI-HF and PstI-HF
(Jeffrey) Picked colonies for 18T entry vectors and miniprepred circuit 2 for test cuts
(Will) Test cut GEX 21-40 and GOP 151-168

MONDAY, 9/12

(Marisa) picked four colonies for 18T g1, g17, g3, g8, and g13
    Digested and test-cut 18T g1, g17, g3, g8, and g13 colonies 1 and 2 with BbsI and SpeI
(Marisa) Ligated and transformed inserts for GEX 3, 5, 8, 10, 15, and 19 into pSB1C3 backbone to create GEX 103, 105, 108, 110, 115, and 119
(Kami) made 8 48 well plates with HEK cells at 100,000 cells/ml
(kami) retransformed pGOP7 because it looked wierd when we transfected it. so going to pick one colony from this transformation to try to start fresh.
(Marisa) Made LB + Cam broth
    Grew up pSB1C3, GOP 35, and GPX2 for midipreps
    Grew up pSB1C3 and GOP 45 (x2) for minipreps

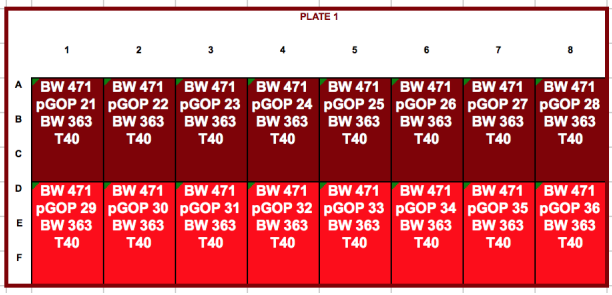
TUESDAY, 9/13

(Marisa) picked two colonies for GEX 103, 105, 108, 110, 115, and 119
    Midiprep pSB1C3, pGPX2, pGOP35
    Miniprep GOP45 (x2), SB1C3, and g17 18T-1/2/3/4
    made master mixes for transfection to take place on 9/14
    picked and grew up a colony from GOP7 re-transformation
    Added Carbacillin to plain LB Broth to make LB+Carb broth
    Grew up Cir4 (x2), SB1C3 (x2), GEX5, GEX 15, GPX2, T40 (x2), and BW471

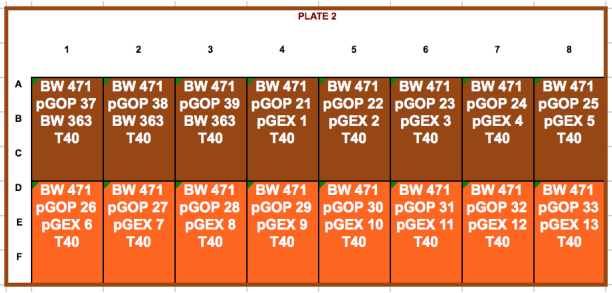
WEDNESDAY, 9/14

(Marisa) miniprepped pGEX 103-1/2, 105-1/2, 108-1/2, 110-1/2, 115-1/2, and 119-1/2
    Test cut GEXs with EcoRI-HF and PstI-HF
    Test Cut g17 18T with BbsI and SpeI
(Marisa and Jeffrey) made 2L of plain LB broth
(Jeffrey) midiprep pSB1C3 (x2), T40(x2), and pCir4(x2)
(Kami Rachel) transfected Hek cells. with pGOPs diluted to 5ng/ul (not 50ng/ul) and add a bigger volume to the transfection mix.

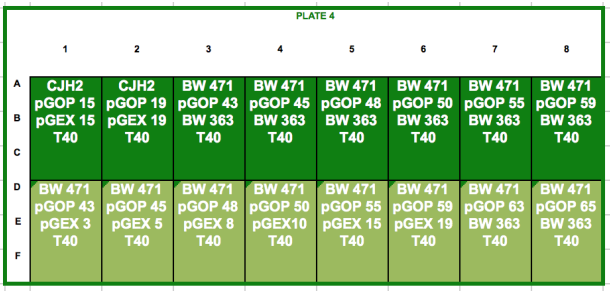
Screen Shot 2016-09-15 at 01.43.39.png



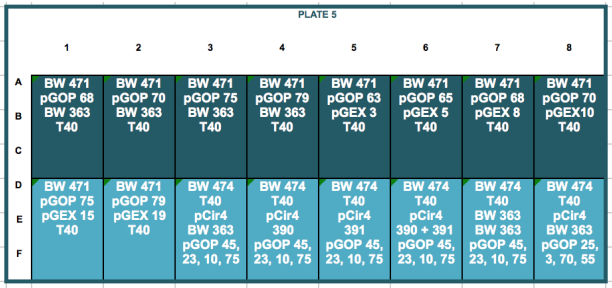
Screen Shot 2016-09-15 at 01.43.46.png



Screen Shot 2016-09-15 at 01.44.02.png

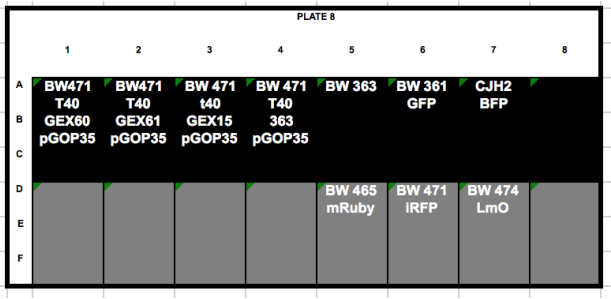


Screen Shot 2016-09-15 at 01.44.20.png



Screen Shot 2016-09-15 at 01.44.29.png

Screen Shot 2016-09-15 at 01.45.21.png



THURSDAY, 9/15

(Marisa) Miniprep GEX 103-3/4/5/6, 105-3/4/5/6, 108-3/4/5/6, 110-3/4/5/6, 115-3/4/5/6, 119-3/4/5/6
Digest and test cut GEXs with EcoRI-HF and PstI-HF
(Marisa and Kami) passaged stable line HEK cells (Wild Type, 2578, and 2579) 1:20
(Jeffrey) Minipreped 1945-1948

FRIDAY, 9/16

(Kami) Miniprep pGOP 159-1/2/3, 160-1/2/3, 169-1/2/3, and 170-1/2/3
(Will) Test cut pGOP 159, 160, 169, and 170

SATURDAY, 9/17

(jeffrey) ligated and transformed BEX34-38

SUNDAY, 9/18

(Marisa and Jeffrey) picked three colonies of GOP 151-158 and 161-168
(Jeffrey) Picked colonies for BEX37 Redigested 1945,1946,1948
(Jeffrey) Religated and transformed BEX 34,35,36,38

MONDAY, 9/19

(Marisa) Miniprep colonies 1,2, and 3 of GOP 151-158 and 161-168
(Jeffrey) PIcked colonies for BEX 34-38
(Kami) Made 6 hek plates at 100,000 (NOT USED)
(Will) Test cut the miniprepped GOPs 151-168

TUESDAY, 9/20

(Marisa) Cell stock BEX 30, GOP 159, 160, 169
(Marisa) made 2L of plain LB broth
(Marisa) Diluted GOP 81-90 to 25 ng/ul
(jeffrey) Miniprepped bex 34-38 and test cut
(Jeffrey) Digested BEX 1,6,15,20, 30,31,32,33
(Jeffrey) digested parts vectors with bbsi for 3 input circuit
(Will) Sent in colony 1 of GOPs 151-168 that test cut correctly

WEDNESDAY, 9/21

(Marisa and Rachel) Made LB agar (x2)
(Marisa) Cell stock pGOP 159, 160, and 169
(Marisa and Kami) Ligated GOP 153, 154, 157, 163, 165, 166, 168, 170, 162, and 167 with respective oigos
(rachel) transformed the pGOPs ligated above
(Kami) Made LB + Cam plates
(Marisa) Made LB + Carb plates
(Jeffrey) Gibson Cir2 and Cir 5 using BEX parts vectors

THURSDAY, 9/22

(Kami) picked 4 colonies for GOP 153, 154, 157, 162, 163, 165, 166, 167, 168, 170
(Jeffrey) Cell stock GOP 151, 152, 155, 156, 158, 161, 164

Miniprepped colonies 5-8 of GOP 153, 154, 157, 162, 163, 165, 166, 167, 168, 170
Test cut GOPs with BbsI and SpeI
Digested BEX 34-37 with AscI and NotI
Digested BEX 38 with AscI and NheI
(Kami) passaged HEK cells
(Will) Sent in colony two of GOPs 151-168 that did not sequence properly

FRIDAY, 9/23

Cell stocked pGOP 154,

SUNDAY, 9/25

(Rachel) Ligated and transformed 1721 insert into MOP (GOP with biobrick cut out)
(Jeffrey) Gibson Circuit 2 and 5

MONDAY, 9/26

(Marisa) miniprepped four colonies of pGOP 104, 109, 135, 137, 144, 149, and 150
Test cut GOP (x3) of 91-100, GOP (x4) 104, 109, 135, 137, 144, 149, 150, 153, 154, 157, 162, 163, 165, 166, 167, 168, 170, and GOP (x3) 113, 135, 136, 138, 142, with BbsI and SpeI
(Marisa) Transformed Circuit 2 and Circuit 5
(Marisa) picked four colonies of MOP + 1721
(Will) Sent in GOP151 and 163 for sequencing
(Kami) made HEK plates

TUESDAY, 9/27

(Marisa) PCR pGEX 3, 8, 15, and 19 and BW 1721
(Marisa and Will) Gel extracted pGEX 3, 8, 15, 19 and BW 1721
(Will) Digested GEX 3, 8, 15, 19 and BW 1721 with EcoRI-HF and PstI-HF
PCR cleanup of GEXs and BW1721
(Rachel) Ligated GEX 3, 8, 15, 19, and BW 1721 with MOP
(Marisa) Transformed GEX 3, 8, 15, 19 and BW 1721 (to GEX 103, 108, 115, and 119)
(Will) Cell stocked pGOP 151 and 163

WEDNESDAY, 9/28

(Marisa) Test cut GOP 162, 163, and 167 colonies 5-8 with BbsI and SpeI
(Marisa) Cell stock 91-100, 104, 109, 113, 136, 137, 138, 142, 144, 149, 150, 153, and 157
(Marisa) miniprep GOP 112, 114, 135,
(Marisa) Replated Circuit 2 and 5

THURSDAY, 9/29

(Marisa) Cell stock pGOP 151 and 163
(Marisa) Replated Circuit 2 and Circuit 5
(Marisa and Rachel) ligated and transformed pGOP 142, 162, and 167

FRIDAY, 9/30

(Marisa) picked 5 colonies of pGOP 167
(Kami) made 11 hek plates at 100,000cells/ul
(kami)Diluted pGOPs to 25ng/ul

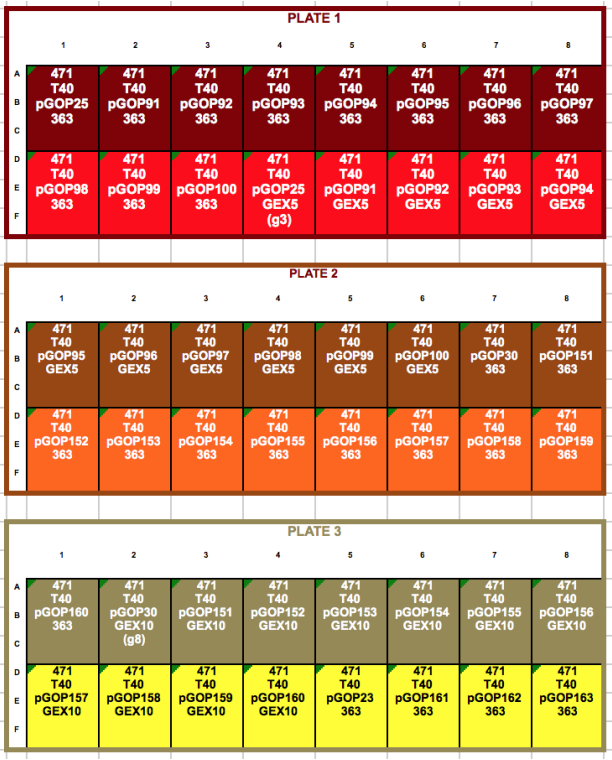
SATURDAY, 10/1

(Marisa) Miniprep and test cut pGOP 167-1/2/3/4/5 with BbsI and SpeI

SUNDAY, 10/2

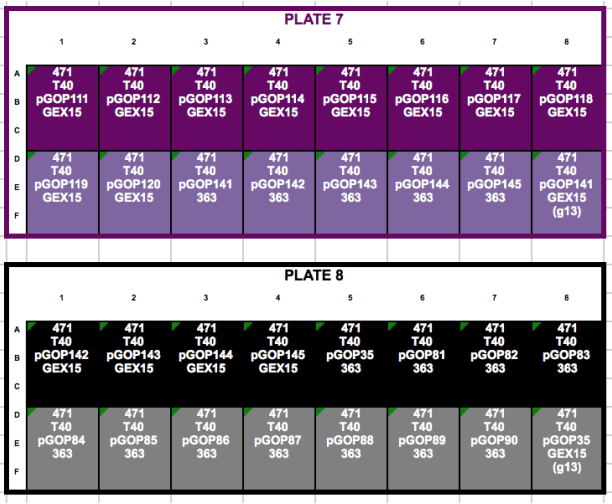
(Kami Rachel) Diluted DNA for transfection.
(Kami Rachel) Transfected hek cells with all the multimerized and mutated pGOPs

Screen Shot 2016-10-10 at 11.34.03.png



Screen Shot 2016-10-10 at 11.34.15.png

Screen Shot 2016-10-10 at 11.34.25.png



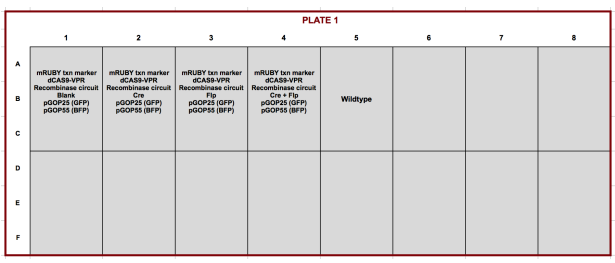
Screen Shot 2016-10-10 at 11.34.37.png

FRIDAY, 10/7

(kami) made 7 hek plates at 100,000 for transfection on sunday and 1 hek plate at 200,000 cells/ml for transfection on saturday with WPI

(Rachel) prepared the transfection DNA mix for WPI

Screen Shot 2016-10-10 at 11.30.05.png



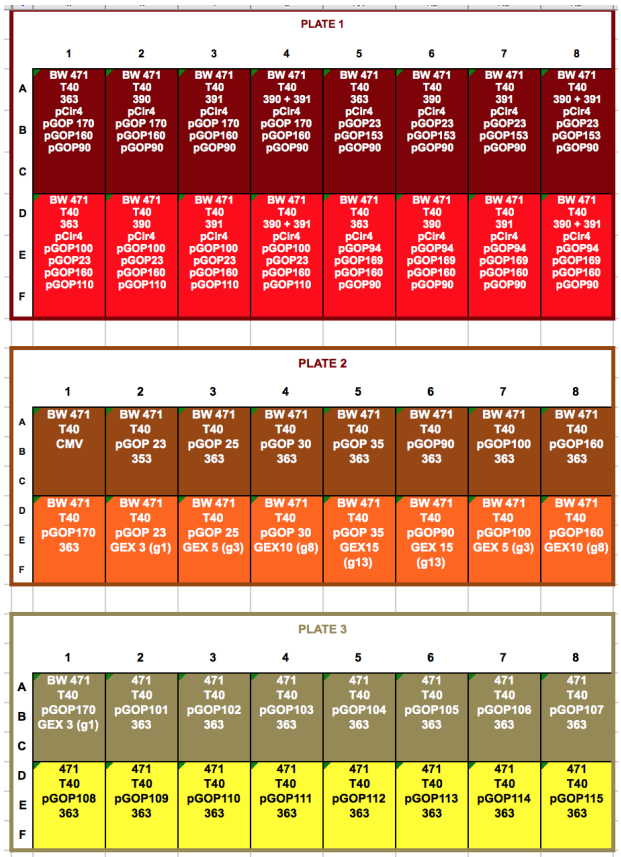
SATURDAY, 10/8

(Kami + Rachel) transfected cells for WPI testing the and/nor circuit
(Kami + Rachel) prepared all the DNA mixes for transfection tomorrow

SUNDAY, 10/9

(Kami) Transfected HEK cells with screen of mutated p GOPs, CMV compared to single and triple multimerized, and 4 analog circuits

Screen Shot 2016-10-10 at 11.32.13.png



Screen Shot 2016-10-10 at 11.32.30.png